THE ONTOGENY OF THE MELANOPHORE CONTROL IN NORMAL COLOUR ADAPTATION IN *XENOPUS LAEVIS*, WITH SPECIAL REFERENCE TO THE PARS INTERMEDIA OF THE PITUITARY

N. ERIK I. NYHOLM

Department of Zoology
University of Lund

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Corrigenda

Title-page:	Chapter III: AN ULTRASTRUCTURAL STUDY OF ACTIVITY ....
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Page I:8:	Second paragraph, seventh line from the bottom: .... Atwell's recess ....
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Page III:3:	Paragraph below the Table; third line should be: 1 % OsO_4 according to Millonig (in Mercer & Birbeck 1966),....
Page III:23:	First paragraph, fourth line: .... development. - should be: .... differentiation.
Fig. III:6:	The figures refer to: 1. Golgi complex, 2. Lysosome, 3. Formation of autophagic vacuole.
Page IV:6:	Text-figure 1b: -MSH - should be (α-MSH)

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Corrigenda

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Page xiii:	Paragraph below the Table: third line should read 1.5 day according to Willsig in Nieuwkoop & Faber (1967).
Page xiii-xiv:	First paragraph, fourth line: ... development should read ... mid-rostration.
Page xiii-xiv:	The figure below Ex. 1. Solid red line: ... transection of endoderm vessels.
Page xiii:	Left-hand ... Pom - should be (Pom-MSH)

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THE ONTOGENY OF THE MELANOPHORE CONTROL IN NORMAL COLOUR ADAPTATION IN *XENOPUS LAEVIS*, WITH SPECIAL REFERENCE TO THE PARS INTERMEDIA OF THE PITUITARY

by N.Erik I. Nyholm

Contents:

To my family:

Sirkka, Charlotta, Annika and Tufve

ABSTRACT

The ontogenesis of the melanophore regulation in early Xenopus laevis tadpoles was studied with regard to the development of the pars intermedia of the adenohypophysis and the participation of the pineal organ.

The first three chapters treat the structural and functional differentiation of the adenohypophysial rudiment, especially the pars intermedia.

I: SOME MORPHOGENETIC FEATURES OF THE ADENOHYPOPHYSIAL RUDIMENT OF EARLY XENOPUS LAEVIS TADPOLES

II: IMMUNOHISTOLOGICAL STUDY OF THE PRESENCE OF MSH AND ACTH IN THE EARLY HYPOPHYSIAL RUDIMENT OF XENOPUS LAEVIS

III: AN ULTRASTRUCTURAL STUDY OF ACTIVITY AND CONTROL OF THE MSH-CELLS IN EARLY XENOPUS LAEVIS TADPOLES

The adenohypophysial rudiment arises at about the developmental stage 20 (Nieuwkoop & Faber) and undergoes a rapid growth. This is arrested from about stage 33/34, when the rudiment also becomes a discrete body and the functional differentiation of the secretory cells starts. During the stages 33/34 to 37/38 the secretory cell population becomes progressively differentiated.

Through a reorganization of the adenohypophysial cells at stage 39, the rudiment becomes subdivided into two compartements - the pars distalis and the pars intermedia. It is suggested that pseudopodia are involved in the process of organization of the cells into the pars intermedia.

The adenohypophysial rudiment is in its "migratory phase" until stage 42. (Chapter I).

With immunohistological technique β-MSH could be demonstrated from stage 37/38 in the caudal half of the adenohypophysial rudiment, and later on, α-MSH as well as

ABSTRACT

The ontogenesis of the melanophore regulation in early *Xenopus laevis* embryos was studied with regard to the development of the pars intermedia of the adenohypophysis and the patterning of the pituitary organ.

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II: SOME MORPHOGENETIC FEATURES OF THE ADENOHYPOPHYSIAL RUDIMENT OF EARLY *XENOPUS LAEVIS* TADPOLES

III: CYTOHISTOLOGICAL STUDY OF THE PRESENCE OF MSH AND ACTH IN THE EARLY HYPOPHYSIAL RUDIMENT OF *XENOPUS LAEVIS*

IV: AN ULTRASTRUCTURAL STUDY OF ACTIVITY AND CONTROL OF THE MELANOCYTES IN EARLY *XENOPUS LAEVIS* TADPOLES

The adenohypophysial rudiment arises as about the developmental stage 20 (Nieuwkoop & Faber) and undergoes a rapid growth. This is separated from mouth stage 23/24, when the rudiment also becomes a discrete body and the functional differentiation of the secretory cells occurs. During the stages 24/25 to 28/29 the secretory cell population becomes progressively differentiated.

Through a reorganization of the adenohypophysial cells at stage 29, the rudiment becomes subdivided into two compartments - the pars distalis and the pars intermedia. It is suggested that pseudopodia are involved in the process of reorganization of the cells into the pars intermedia.

The adenohypophysial rudiment is in its "secretory phase" until stage 42. (Chapter 1).

With immunohistochemical techniques, MSH could be demonstrated from stage 27/28 in the ventral part of the adenohypophysial rudiment, and later on, ACTH as well as

/3 - MSH in the same cells of the pars intermedia. It is suggested that MSH is produced in the rudiment also at earlier stages, but then occurs in too low amounts to be detectable with the technique used. ACTH could not be demonstrated in the rudiment at the stages studied, to stage 46.(Chapter II).

The functional differentiation of the MSH-cells starts at about stage 33/34. It is accompanied by an increased development of the rough endoplasmic reticulum (RER), Golgi complexes, and an increase in the population of mitochondria and with rapidly decreasing amounts of yolk material. Increased development of the RER and the Golgi complexes indicated progressively increased MSH synthesis in all the tadpoles to about stage 40. After that stage, specimens which are kept on a white background deviate from those on a black by decreasing RER, which indicates decreased MSH synthesis. Simultaneously MSH release decreases as suggested by pile up of secretory granules in the cells.

The decrease of yolk and the increase of the mitochondrial number seem partly to be associated which each other. The yolk platelets seem to represent two types. In one of these, which is in minority, mitochondria are suggested to be formed.

Soon after its formation, the pars intermedia at advanced stage 39 becomes innervated by monoaminergic nerves which originate in the CNS. Only this nerve-type has been observed within the rudiment at the stages studied, to stage 43. The appearance of these nerves in the pars intermedia coincides with the ability of the tadpoles to inhibit synthesis and release of MSH when kept on a white background. This control is suggested to be functionally associated with the monoaminergic nerves. At stage 43 also peptidergic nerve profiles were observed, but only in the infundibular floor. (Chapter III).

The differentiation of the MSH-cells and their control, as reflected in the activity of the melanophores, is the

activity of the melanophores, is the subject of the fourth chapter.

IV: THE DEVELOPMENT OF THE MELANOPHORE CONTROL IN EARLY XENOPUS LAEVIS TADPOLES

At about stage 33/34 the first melanophores appear. This coincides with the first appearance of secretory activity in the adenohypophysial rudiment. During the further development, to advanced stage 39, the melanin of the melanophores disperses irrespective of the background colour. This dispersion is conditioned by MSH release from the adenohypophysial rudiment, as it is abolished by hypophysectomy and regained after re-implantation.

In association with the innervation of the pars intermedia, at advanced stage 39, the tadpoles obtain the ability to adapt the melanophore states according to the background colour.

In transplanted adenohypophysial grafts the pars intermedia became secretory active at the same stage as in normal tadpoles and without direct contact with brain tissue. This suggests that the MSH-cells, once determined - are self-differentiating.

Besides by the pars intermedia of the hypophysis, the melanophore regulation in amphibians has been proposed to be influenced by the pineal organ. This aspect is tested in chapter V.

V: AN EXPERIMENTAL STUDY OF THE ROLE OF THE PINEAL ORGAN IN THE CHROMATIC RESPONSES IN EARLY XENOPUS LAEVIS TADPOLES

It was revealed that the chromatic responses - the"body blanching reaction" and the background response - became abolished after pinealectomy, but only temporarily. A rapid re-establishment (in 2 to 3 days) of the responses indicates that the pineal organ is not of decisive importance for the chromatic responses in amphibians.

INTRODUCTION

Many poikilothermic vertebrates are able to change their skin colour in respo se to environmental stimuli. This colour change is carried out by migration of pigment within specialized cells (chromatophores) in the skin (Parker 1948, Waring 1963, Burgers 1966, Bagnara & Hadley 1973).

Four types of chromatophores can be distinguished (Bagnara & Hadley 1973): Melanophores, containing black or brown melanins; iridophores (leucophores, guanophores) which reflect light; the yellow xantophores and the red erythrophores.

A variety of factors may influence the process of pigment migration, as environmental light, temperature and humidity, and also the psychic state and the internal rythm of the animal. The pigment migrations usually are not accom--lished by direct influence of these factors upon the chromatophores. Exceptions excist however; one of these is the direct influence of light upon tail melanophores in advanced Xenopus laevis tadpoles in which light induces melanin concentration (Bles 1905, Bagnara 1957, van der Lek 1958).

The chromatophore responses are mainly controlled by hormones.

Adrenalin generally causes melanin concentration in fish (Fuji 1961), amphibians (Zimmerman & Dalton 1961) and reptiles (Kleinholtz,1938). In Xenopus laevis, however, adrenalin may induce melanin dispersion (Burgers 1956).

Melatonin is a very potent melanin concentrating agent which most probably is involved in the "body blanching reaction", as thoroughly studied by Bagnara (1965) in Xenopus laevis tadpoles. This reaction involves melanin concentration when the tadpoles are transferred to darkness.

The most important chromatophore controlling hormone is

the melanophore stimulating hormone (MSH) which is produced by the pars intermedia of the hypophysis. This hormone is responsible for melanin dispersion in the melanophores and does also control the activity of iridophores in amphibians (Bagnara 1958).

The background response involves pigment migrations influenced by changes of the background colour, leading to colour adaptation. Usually this means that the animal becomes dark when kept on a dark background and light when on a light background. The dark colour is mainly conditioned by melanin which is dispersed by the action of MSH. The chromatic adaptation to a light background is mainly brought about by concentration of the melanin in the melanophores. In teleost fish, except eel, the melanin concentration is induced by nerves (Jacobovitz & Laties 1968, Falck et al. 1969). In elasmobranchs, amphibians and Anolis (Reptilia) the colour adaptation to a light background is hormonally controlled and depends, in part at least, upon the release of MSH being inhibited. Also the occurrence of a special whitening hormone is suggested in amphibians. (For further references on background response, see Parker 1948, Waring 1963, Bagnara & Hadley 1973).

The MSH-release from the amphibian pars intermedia of the hypophysis is controlled by inhibitory nerves of hypothalamic origin (Etkin 1941, see further in review by Terlou et al. 1974).

The present study treats the ontegeny of the melanophore controlling mechanism in Xenopus laevis tadpoles. Mainly concerned are the differentiation of the activity and control of the pars intermedia.

The amphibian species used in this study, the south African Clawed toad, Xenopus laevis (Daudin) is a representative of the anuran family Pipidae.

This species has become well known in many countries since it for a long time was used as a test material for human

pregnancy. It also turned out to owe other favourable qualities and is, as tadpoles and adults, still used in several laboratories all over the world for physiological research. Thus, for example, the rearing methods are simple and breeding is easily induced at any time of the year.

The Xenopus tadpoles are especially well suited for research on chromatic responses. As a contrast from, for example tadpoles of European amphibians, the pigment , mainly melanin, of Xenopus tadpoles is almost exclusively bound to chromatophores. In the tadpoles of European amphibians the chromatophores are mostly covered by free melanin of the epidermal cells, which under natural conditions gives the always dark appearance of these tadpoles. The chromatophore-bound pigment of the Xenopus tadpoles gives these the possibility to colour adaptation, according to the environmental conditions, from relatively early developmental stages. The biological significances of the different features of pigmentation has to my knowledge not been studied, but should prove an interesting research subject.

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Chapter I

SOME MORPHOGENETIC FEATURES OF THE ADENOHYPOPHYSIAL RUDIMENT OF EARLY *XENOPUS LAEVIS* TADPOLES

ABSTRACT

The adenohypophysis of *Xenopus laevis* tadpoles of the developmental stages 20 to 46 (Nieuwkoop & Faber 1956) were studied. From its arisal at about stage 20 to 21 the adenohypophysial rudiment passes through four morphogenetic phases, each characterized by internal events. The first phase (stage 20 to about 33/34) is characterized by extensive proliferation of the rudiment. During the second phase (stage 33/34 to about 37/38) the growth of the rudiment is arrested and the shape is constantly claviform. The morphogenetic stand-still coincides with the attainment of secretory function of the adenohypophysial cells. The third phase, which roughly comprises stage 39, is characterized by a thorough reorganization of the adenohypophysial cells, leading to the formation of the pars distalis and pars intermedia. The shape of the rudiment changes and its volume temporarily increases. The last phase is characterized by the organization of the pars distalis cells into cell cords with possible functional relations with a specialized region (in this paper called the "hilus") of the adenohypophysis - brain interspace. The homology of the "hilus" with Atwell's recess of amniotes is tentatively suggested.

INTRODUCTION

The morphogenesis of the amphibian adenohypophysis has been the subject of several studies: Atwell (1921), Sumi (1924; 1926), and Copeland (1943) in urodeles; Atwell (1918) in *Rana temporaria*, Schliefer (1935) in *Bufo bufo*, Nieuwkoop & Faber (1956) and Frick (1967) in *Xenopus laevis*. The development of the adenohypophysis is by these studies shown to be similar, gross-morphologically, in the different amphibian species during the embryonal

stages. The internal morphogenesis of the adenohypophysial rudiment seems, however, largely to have been neglected by the above mentioned authors.

The present study of the adenohypophysial morphogenesis of Xenopus laevis tadpoles concerns some external and internal features of the rudiment which are considered to be associated with the differentiation of its glandular function.

MATERIAL & METHODS

The tadpoles were obtained from adult Xenopus laevis (Daudin) in which spawning was induced by HCG (Gonadex, Leo, Sweden) injected into the dorsal lymph. sac. The dosage, about 600 I.U. in the females and about 300 I.U. in the males, was given as a single dose in accordance with Henriques (1964).

The offspring was kept in room temperature and classified into developmental stages according to Nieuwkoop & Faber (1956).

Tadpoles were fixed intact in Bouin-Holland sublimée. Dehydration was performed in tetrahydrofurane, paraffin sections (5-6 m) were stained with Azan or Ehrlich's haematoxylin-eosin.

The methods used for the ultrastructural preparations - see Material & methods, chapter III.

For the volumetric estimations of the adenohypophysial rudiment, the outlines of sections of the organ were drawn by means of a camera lucida on paper with uniform thickness and quality. From the weight of the cut out drawings, volume indices were obtained. These were statistically tested with the Student's t-test.

RESULTS

The adenohypophysial primordium of Xenopus laevis tadpoles appears during the stages 20 to 21 as a compact wedge-like bud arising from the inner layer of the skin-ectoderm in the dorsal region of the oral invagination. Still attached to this ectodermal layer until stage 32 to 33/34, the bud extends caudally between the brain and the foregut, and becomes claviform. Thereafter the attachment breaks and the adenohypophysial rudiment "migrates" further caudad as a discrete body. The "migration" occurs close to the brain floor.

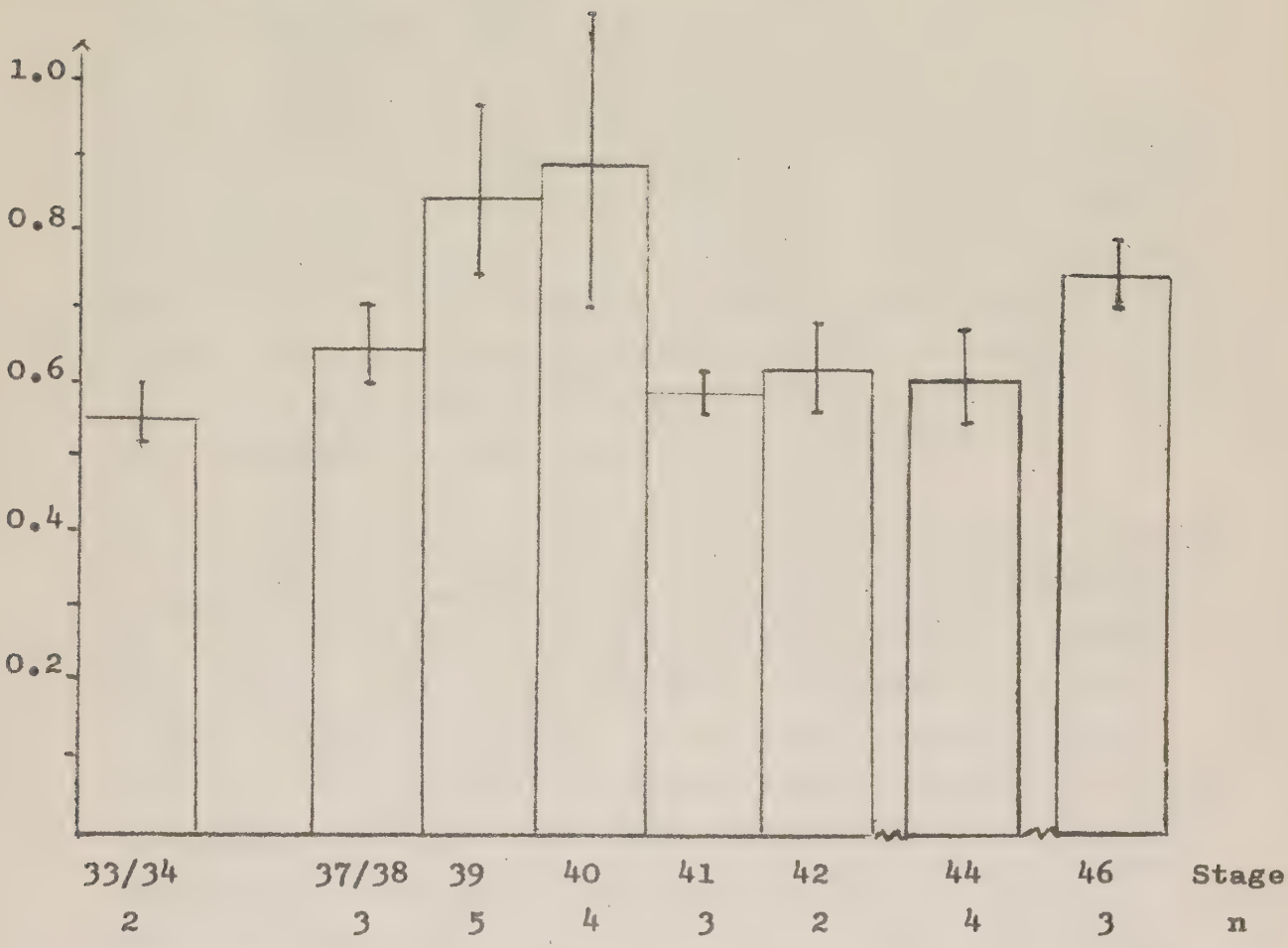
At stage 33/34 the caudal tip of the rudiment has reached the caudal end of the chiasmic ridge (fig. 1). From stage 35/36, a progressively larger part of the rudiment passes over onto the thin infundibular floor (fig. 2). When the "migration" of the hypophysial rudiment is completed at stage 42, it borders the infundibular floor along its whole length (fig. 3). The caudal end of it then borders the tip of the notochord.

After about stage 37/38 the shape of the adenohypophysial rudiment undergoes a marked change. From being claviform during the earlier stages (fig. 4), it broadens in the lateral and dorso-ventral directions simultaneous with a shortening of the fronto-caudal axis (figs 5, 6, 7).

Along with the change of the shape of the adenohypophysial rudiment, its volume increases temporarily, to achieve relatively high values at the stages 39 and 40, followed by a decrease to the original valus. (Text fig).

Text-figure

Volume of the
adenohypophysial rudiment
(Index values)



The volumes of the stages 39 and 40 are significantly different from the others: t = 7.602, p 0.001.

The process of change of the outer shape is associated with an internal cellular reorganization which concerns all regions of the rudiment. This reorganization leads to the formation of two compartments of the rudiment which become distinguishable during stage 39, from their different cellular arrangements. In the dorso-caudal part, which immunohistologically is defined as the pars intermedia (cf chapter II), the cells seem to be rather randomly arranged (figs 5, 6, 7).

An ultrastructural examination reveals some special features of the prospective pars intermedia cells which may be of significance for the process of the formation of that lobe. Thus these cells frequently are provided with far-reaching slender processes which seem to be directed mainly along the fronto-caudal axis of the rudiment (fig. 8). The actual role of these processes is not revealed by the present study, but they may be suggested to represent pseudopodia. Cell processes, which originate from MSH-cells are frequently observed in the pars intermedia from stage 39, sometimes forming large conglomerations (Chapter III, fig. 10). It seems reasonable to assume that these cell processes correspond to those of the prospective pars intermedia cells, observed at stage 37/38.

In the frontal compartment of the adenohypophysial rudiment - which con sequently mainly represents the pars distalis - the cells, from stage 39, progressively become arranged into columns (figs 5, 6, 7). These essentially converge against a distinct region, on the brain bordering side, where the outline of the rudiment forms a shallow invagination (figs 5, 6, 7). Secretory cells of the pars distalis, which are distant from this invagination, develop processes which penetrate on to it. Secretory granules are transported in these processes and obviously stored in distal swellings of them. In the invagination - between the adenohypophysis and the brain - the first capillaries to be observed in the hypophysial region are differentiated out of fibroblasts, from about stage 43 (fig. 9).

The region where the adenohypophysial border invaginates may be regarded as a "hilus" formation to which secretory products of the pars distalis are transported and possibly released into the capillaries.

DISCUSSION

The adenohypophysial rudiment of Xenopus laevis arises at stage 20 to 21 as a wedge-like bud. The further development involves that the rudiment undergoes a morphogenesis which during the stages studied, to stage 46, may be subdivided into four phases.

The first phase extends from stage 20 to 21 until about stage 33/34. The duration of this period is about 24 hours. This first phase is characterized by an extensive growth of the adenohypophysial rudiment, which, still attached to its mother-tissue, extends caudally along the brain floor and reaches behind the optic chiasm.

The second phase begins at about stage 33/34, when the rudiment looses its attachment to the frontal ectoderm and initiates its caudad "migration" as a discrete body. The end of this phase is set to about stage 37/38, which means that the duration will be about 12 hours. The outline of the rudiment does not change much during this phase - the shape of it is claviform and the volume seems to be fairly constant. Instead this phase exactly coincides with those developmental stages during which the secretory function of the rudiment becomes differentiated as revealed by ultrastructural evidence (cf chapter III). Arrested growth of the adenohypophysial rudiment when attaining its secretory function is also observed in Ambystoma-species (Rhodes & Dalton 1961).

The third phase approximately comprises stage 39. The duration is about 10 hours. This phase is characterized by the thorough reorganization of the adenohypophysial cells which leads to the formation of the pars distalis and the pars intermedia (cf chapter II). In the process of cellular reorganization, especially the prospective pars intermedia cells seem to take an active part. Thus processes of these cells are frequently observed at stage 37/38, which may represent pseudopodia promoting the active migration. After the formation of the pars intermedia, these processes probably are retained, but

then probably with a role concerned with the secretory activity of the cells (cf chapter III). As far as observed the pars distalis cells do not form cell processes to any noticable extent during this stage. In the amphibian embryo gastrulation is suggested to be at least partly brought about by active migration of individual cells by means of pseudopodia (Nakatsuji 1974). Pseudopodia were observed to be formed by endo-, meso-, as well as ectodermal cells.

The increase of the volume of the adenohypophysial rudiment from stage 37/38 to 39 and 40 may at least partly be associated with the process of cellular reorganization, during which the cells seem to become relatively loosly arranged.

During this - the third - phase, the adenohypophysial rudiment, while still "migrating", becomes innervated by nerves penetrating into it from the infundibular floor (cf chapter III). These facts suggest that during the last part of the "migratory" periods the rudiment is anchored to the infundibular floor, which then in some way is responsible for the dynamics of the migration.

A forth phase comprises the developmental stages from about 40. During this phase the pars distalis cells progressively become organized into cell cords. These cords converge against the region called the "hilus" in this paper. The cell bodies of the pars distalis which are distant from that region send processes to reach it. In the cell processes secretory material is transported to be stored in distal swellings of the processes. As to further stress the significance for the secretory activity of the pars distalis, the "hilus" is the site for the differentiation of the first capillaries observed in the hypophysial region.

The volume of the adenohypophysial rudiment undergoes a decrease from the temporary high values during the stages 39 and 40, This decrease brings the volume back to the same values as before stage 39, which suggests that the mitotic activity of the adenohypophysial cells essentially is regressed during the last three morphogenetic

phases of the present study. The progressively closer arrangement of the pars distalis cells may support the volume decrease.

The adenohypophysial bud, though compact, is to be considered homologe with Rathke's pouch of the amniotes. Besides from the pars distalis, pars intermedia and pars tuberalis in the adults, further homologization with the amniote adenohypophysis seems not to have been suggested (cf Wingstrand 1966). The "hilus" of the adenohypophysis, as described in this paper may however be interesting also in the respect of homology: At about stage 37/38 mesenchymal cells invade, forming a thin sheeth, between the adenohypophysial rudiment and the brain. In a limited region the distance between these two organs become widened through the formation of a shallow mesenchyme filled invagination of the adenohypophysial wall. This invagination is retained during the further stages studied. After the cellular reorganization of the rudiment the invagination is surrounded by pars distalis tissue. The mesenchymal sheeth is interpreted as representing pial mesenchyme. In the invagination ("hilus"), this mesenchyme is the origin of capillaries as observed in the Xenopus tadpoles from stage 43. The way and site of the formation of the "hilus" may tentatively be suggested to correspond to Atwelll's recess of the amniote adenohypophysial rudiment (cf Enemar 1960). If so, the pial capillaries should be associated with the pars distalis. This seems to be the case, but instead of the capillaries penetrating into the rudiment, the cells of pars distalis reach the vicinty of the capillaries through extensive process formation.

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Figures I: 1 - 3.

Figure 1-3a. Frontal part of *Xenopus* tadpoles in median sagittal view.

Figure 1-3b. Brain base and adenohypophysial rudiment.

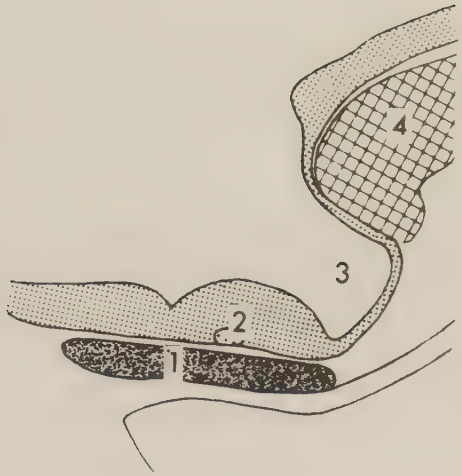
Fig. 1a,b Stage 33/34

Fig. 2a,b Stage 37/38

Fig. 3a,b Stage 42

1. Adenohypophysial rudiment
2. Optic chiasm
3. Third ventricle (infundibulum)
4. Notochord

a



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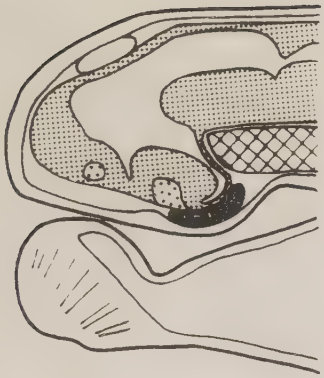

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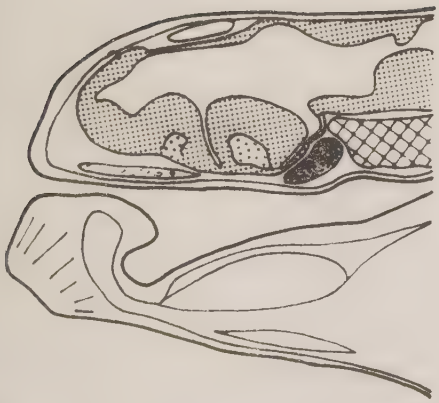

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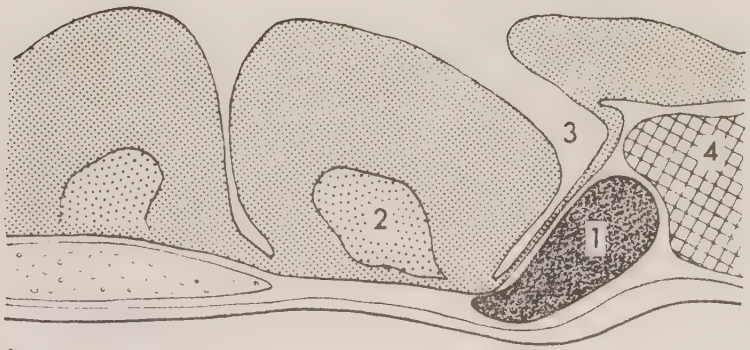

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Figures I: 4 - 7.

Figures 4 - 7. Median sagittal view of adenohypophysial rudiment. The nuclei of the hypophysial cells indicated.

Fig. 4 Stage 37/38

Fig. 5 Stage 40

Fig. 6 Stage 43

Fig. 7 Stage 46

1. Third ventricle (infundibulum)
2. Infundibular floor
3. Site of invagination of the adenohypophysial rudiment
4. Pars intermedia
5. Pars distalis

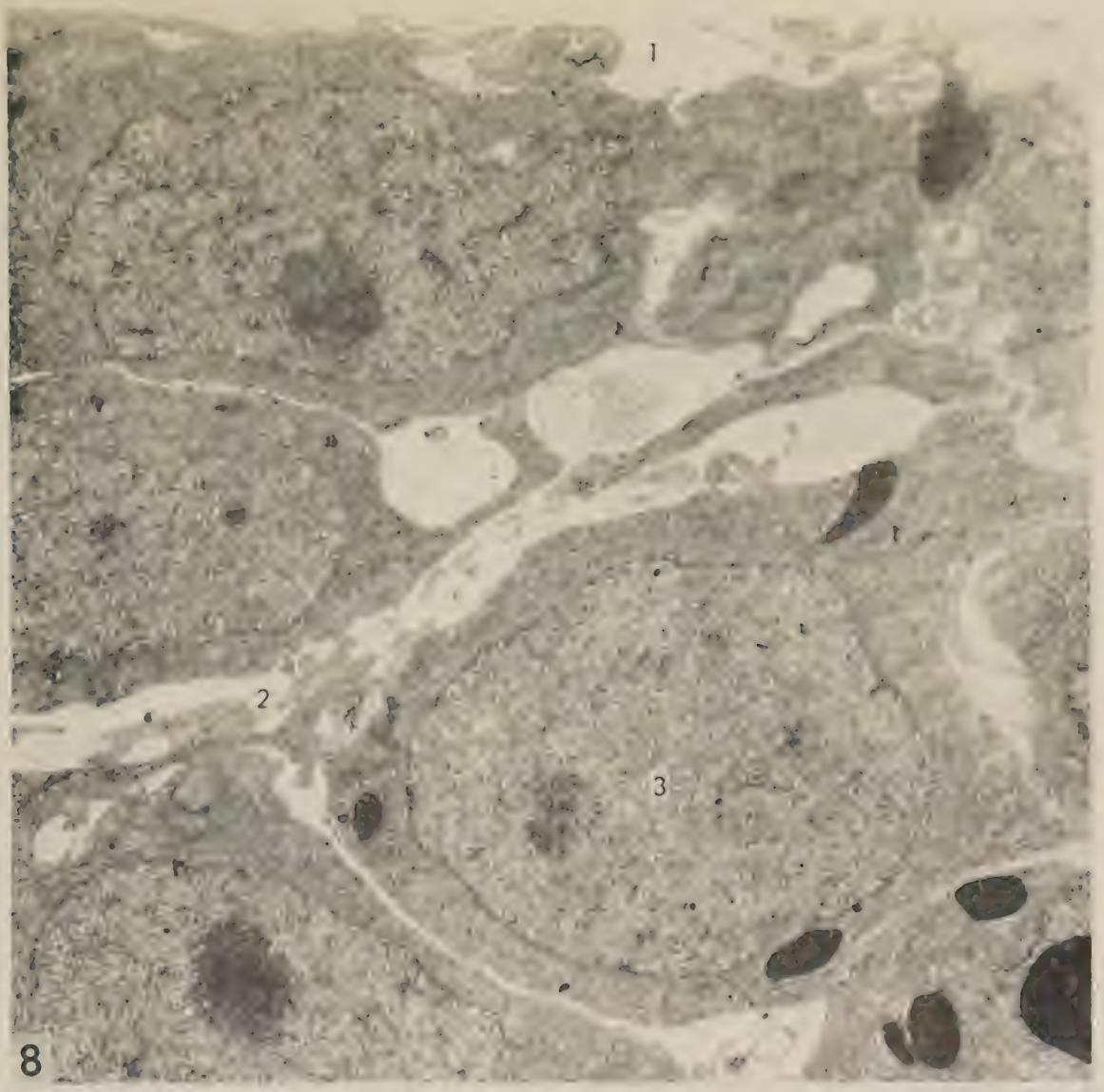
Figures I: 8 - 9.

Figure 8. Portion of the caudal part of the adenohypophysial rudiment.
Stage 37/38, 8 400 X.

1. Hypophysis - brain interspace
2. Cellprocesses
3. MSH-cell

Figure 9. "Hilar" region of the adenohypophysial rudiment.
Stage 43. 4 700 X.

1. Infundibulum
2. Nervebundle in the infundibular floor
3. Fibroblast differentiating into capillary
4. Distal swellings of pars distalis cell processes, with secretory granules.
5. Pars distalis cell processes with secretory granules.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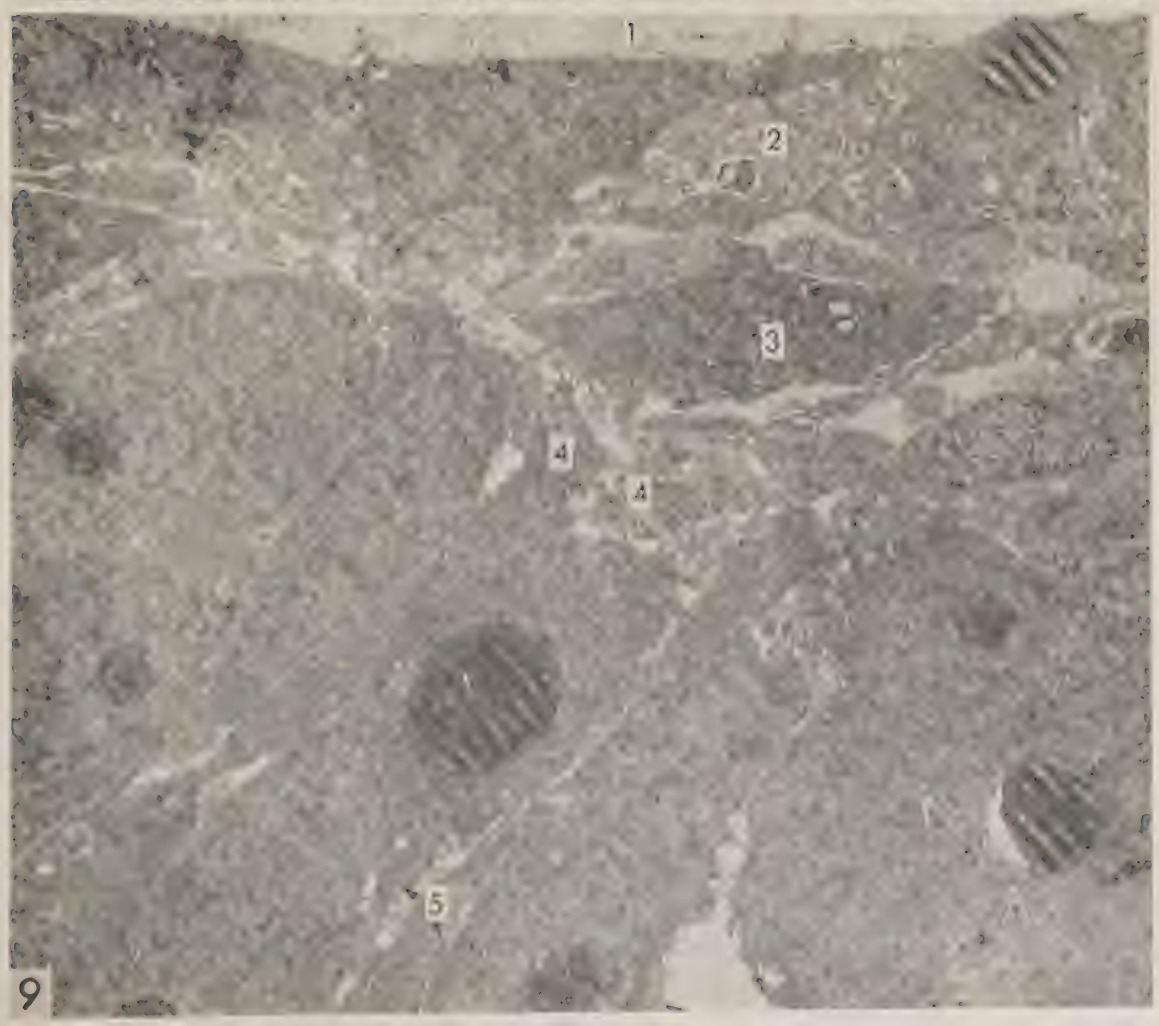
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Chapter II

IMMUNOHISTOLOGICAL STUDY OF THE PRESENCE OF MSH AND ACTH IN THE EARLY HYPOPHYSIAL RUDIMENT OF *XENOPUS LAEVIS*

ABSTRACT

The occurrence of α-MSH, β-MSH and ACTH in the adenohypophysial rudiment of *Xenopus laevis* tadpoles of the developmental stages 33/34 to 46 (Nieuwkoop & Faber 1956) were tested for with immunohistological technique.

β-MSH could be demonstrated from stage 37/38, and α-MSH from stage 39. No indications of ACTH production were obtained. α-MSH and β-MSH occur in the same cells. No differences could be observed in the intensity of the immunofluorescence between tadpoles which were kept on black or white background.

The hypothesis concerning the derivation of α-MSH from ACTH (see Lowry & Scott 1975) is not supported by the present study.

The specific identification of the MSH-cells made it possible to follow the course of pars intermedia formation. This is considered to be accomplished during the stage 37/38 to 39. From a strandlike arrangement of the MSH-cells at stage 37/38, they are organized into a cluster (pars intermedia) in the dorso-caudal part of the hypophysial rudiment at stage 39.

INTRODUCTION

In the previous chapter the morphogenesis of the early adenohypophysial rudiment in *Xenopus laevis* was described, and the thorough reorganization of the cells during the developmental stages 37/38 to 39 was discussed. This reorganization leads to a transformation of the outer shape of the rudiment and to an internal separation into two compartments which are characterized by different cellular arrangements.

The compartments are located in the positions of the pars intermedia and pars distalis. The functional differentiation of hypophysial cells for production of melanophore active substance(s) (Etkin 1941; Pehlemann 1962, 1965; Nyholm 1969, 1972) is, however, achieved earlier than the above-mentioned morphological differentiation.

The identification of the melanophore active substance(s), and the localization of the cells that produce it, would enlighten the discussion about the functional and morphological differentiation of the hypophysial rudiment.

To achieve this, the presence and localization of those melanophore active substances that are known to occur in the amphibian hypophysis - MSH and ACTH - were studied with immunohistological technique. This technique has been successfully employed by Doerr-Schott & Dubois (1972, 1973) and Doerr-Schott (1974) in the amphibian hypophysis.

MATERIAL AND METHODS

Tadpoles of Xenopus laevis Daudin were obtained from adults in which spawning was induced by injection of HCG[x)] into the dorsal lymph sac. The female and male were given about 600 I.U. and 300 I.U. respectively, as a single dose (Henriques 1964).

The tadpoles were kept at room temperature.
One tadpole series was kept on an illuminated white background and a second series on a black background, from developmental stage 20/21 until fixation. In a third series the tadpoles were kept on a black background until 30 minutes before fixation when they were transferred to a white background.

The total numbers of specimens of different developmental stages (Nieuwkoop & Faber 1956) are shown in Table II:1.

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x) Gonadex, Leo, Sweden

Table II:1 The numbers of investigated specimens of different developmental stages.

Stage:	33/34	35/36	37/38	39	39/40	40	41	42	43	44	45	46
Numbers:	3	10	10	17	15	16	15	22	13	4	6	12

The tadpoles originated from two broods.
They were fixed in Stieve (Romeis 1968 § 333) or in Bouin-Hollande (Romeis 1968 § 303) with 10 % saturated sublimate added, (BHS).

The specimens were dehydrated in tetrahydrofuran or alcohol-xylol, embedded in paraplast and sectioned 6 µm.

The antigenes used were: Synthetic mammalian α-MSH, synthetic bovine β-MSH, β (1-24)ACTH (synacthen) and α (17-39)ACTH.[x)]

To make these peptides more antigenic, they were coupled to an albumine (ovalbumine, human or bovine serum albumine, Vance et al 1968). For the preparation of the antibodies, the peptide-albumine complex was administered into rabbits, together with the complete Freund's adjuvant. This preparation and the serological tests of the antibodies were performed by Dubois (Dubois 1971; Stefan & Dubois 1972).

For the immunohistological reaction, the antibodies were appropriately diluted with a solution of veronal buffer (0,1 M, pH 7.2-7.6) and 2 % albumine of the same kind as that incorporated in the antigen-complex. The albumine is added in order to saturate sites which could unspecifically fix the albumine-part of the antibody.

The fluorescence was obtained with isothiocyanate of fluorescein which was conjugated with sheep anti-rabbit-immunoglobuline.[xx)]

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x) Ciba laboratories, Bale

xx) Louis Pasteur institute, Paris

The specimens were finally counter-stained with Evans blue (1:10 000) to obtain a more accentuated contrast between fluorescent and non-fluorescent cells.

The following control tests were performed:

a) The antibodies were omitted in the procedure: No fluorescence developed.
b) Inhibition tests on the antibodies using homologous and heterologous antigenes. From table II:2 it can be seen that the activity of the antibodies was totally inhibited by the homologous but unaffected by the heterologous antigenes.

Table II:2 Inhibition tests of the activity of the antibodies with homo- and heterologous antigenes.

Antibody	Antigenes			
	α-MSH	β-MSH	β (1-24)ACTH	α (17-39)ACTH
Anti-α-MSH	0	+	+	+
Anti-β-MSH	+	0	+	+

0 No fluorescence
+ Fluorescent intensity unaffected

Sections which had passed through the immunohistological procedure were later stained with azan.

RESULTS

In Xenopus tadpoles of the developmental stages 33/34 to 46 no indication of ACTH production, in the hypophysial rudiment, was obtained, neither with the anti-β (1-24)ACTH nor the anti-α (17-39)ACTH antibodies. That this result dit not depend on a low activity of the antibodies used, was clear as when parallelly tested on pituitaries of adult Xenopus, a bright fluorescence was exhibited by the corticotropic cells of the pars distalis and by the pars intermedia cells (cf. Doerr-Schott & Dubois 1972).

The anti-β-MSH serum evokes immunofluorescence in the cytoplasm of hypophysial cells from stage 37/38 (figure 1a) whereas the presence of α-MSH can be demonstrated slightly later, in stage 39. The same results are obtained in tadpoles after the different fixations and background histories.
The two MSH:s are present in the same cells.
The intensity of the β-MSH fluorescence is relatively low during the stages 37/38 and 39 but reaches a higher level from stage 40, figures 1a-5a. This is the case in all tadpoles, in different fixatives as well as with the different background histories. Likewise an increase of the α-MSH-fluorescence occurs from stage 40.

The numbers of MSH cells seem to be rather constant during the stages 37/38 to 46.

The distribution of the MSH-producing cells is markedly shifted as the transformation of the hypophysial rudiment occurs, between stages 37/38 and 39/40 (cf. this paper chapter I). During the earlier stage the MSH-cells are arranged in a strandlike fashion through the caudal half of the hypophysis (figure 1b). The transformation of the hypophysial rudiment implies a thorough reorganization of all the hypophysial cells among which the MSH-producing cells become grouped in their definite position as a pars intermedia (Fig 2a-5a). The MSH-cells get rather loosely arranged in a seemingly irregular pattern. This is in contrast to the pars distalis cells which are successively oriented into strands which are directed towards the "hilar" region, figures 2b-5b.

DISCUSSION

The relevance of using anti-mammalian-MSH (and ACTH) antibodies to immunologically identify and localize amphibian MSH (and ACTH) rests on the assumption that the hormones are chemically very closely related in the two vertebrate groups. Electrophoretic studies of the intermediate lobe extracts of Rana catesbiana indicate the presence of α-MSH, β-MSH and a third compound with MSH-activity (Burgers 1960). MSH:s in Rana pipiens behave identically

to mammalian α- and β-MSH and allow quantification against standards of these hormones, in polyacrylamide electrophoresis (Preslock & Brinkley 1970). Also in Rana pipiens a third melanocyte stimulating substance has been found. These electrophoretic properties indicate the very close relationship between mammalian and amphibian MSH, which makes it very reasonable to believe that the results obtained in the present study show the true identity of α- and β-MSH and the localization of the production sites in Xenopus tadpoles. Minor differences in the chemical structures might, however, occur and to an unknown extent affect the readiness of the fixation of the anti-mammalian-MSH antibodies to the amphibian MSH in the tissue, and possibly limit the sensitivity of the technique.

The failure to demonstrate ACTH in the hypophysial rudiment of the Xenopus tadpoles may mean that this hormone is not produced, or is produced in too small quantities to be detected, during the developmental stages in question. A different explanation could be that the used anti-ACTH antibodies do not fix to the Xenopus ACTH in the tissue. This is, however, contradicted by the fact that these antibodies were fixed in the corticotropin cells in adult Xenopus hypophyses which were treated parallelly with the hypophysial rudiments of the tadpoles. In the adult the immunofluorescence occurred in a rostral region of the pars distalis and in most cells of the pars intermedia. This is the same distribution of the ACTH-producing cells as previously described in Xenopus laevis, Rana temporaria and Bufo vulgaris (Doerr-Schott & Dubois 1972) and urodeles (Doerr-Schott 1974). The identity of the fluorescent cells of the pars distalis as corticotrophs is confirmed by experiments with interrenalectomy in Rana esculenta (Doerr-Schott 1972, a,b).

The fact that also α(17-39)ACTH antibodies give immunofluorescence in the adult pars intermedia cells should eliminate the idea of cross-reaction between anti-ACTH antibodies and MSH, as it has no amino acid sequence in common with any MSH molecule. For references see review by Moriarty (1973), and Dupouy & Dubois (1975).

In 10 mm tadpoles of Rana temporaria, Doerr-Schott & Dubois (1973) found that anti β(1-24)ACTH as well as anti-α(17-39) ACTH were fixed in the cells of the rostral part of the hypophysis. It is hazardous to compare the stage of development in tadpoles of different species but judging from fig. 1 in the mentioned publication, the development of the hypophysis of the 10 mm Rana temporaria tadpole seems to be comparable with that of Xenopus during stage 46 to 47. ACTH production thus may start earlier during the development in R. temporaria than in Xenopus. The occurrence of demonstrable amounts of ACTH in the pars distalis of the 10 mm R. temporaria hypophysis seems, however, contradictory to the results by Doerr-Schott (1968). In an ultrastructural examination of the 10 mm tadpole she found that the hypophysial cells, which contained secretory granules, from their localization might be classified as pars intermedia cells. This descrepancy probably depends on body length of the tadpole being a rather unaccurate criterion for developmental stage.

β-MSH occurs in the hypophysial rudiment of Xenopus, in quantities which are demonstrable with the immunohistological technique, from stage 37/38. α-MSH is present at the slightly older stage 39.

From hypophysial transplantation experiments in various amphibians including Xenopus, the melanophore-stimulating activity is found to be the earliest demonstrable function of the hypophysial rudiment (Pehlemann 1962, 1965). From differences in pigmentation in normal and hypophysectomized Xenopus, Etkin (1941) concluded that the hypophysial rudiment secretes melanophore-active substance from about 48 hours after fertilization. This is in accordance with results obtained from similar experiments by Nyholm (1969) who found such pigmentation differences from stage 33/34. An ultrastructural investigation reveals the appearance of the earliest secretory granules in the hypophysial rudiment in Xenopus tadpoles during the same stage (Nyholm 1972, this paper chapter III).

There is thus a discrepancy, which seems considerable, between the time for the onset of the secretion of melanophore-stimulating substance, as experimentally determined, and the earliest demonstration of MSH with the immunohistological technique. The corresponding difference seems to be of the same order in Rana temporaria tadpoles (Pehlemann 1965, Doerr-Schott & Dubois 1973).

As the immunohistological technique did not visualize ACTH during the developmental stages which were investigated, 33/34 to 46, that hormone is with reasonable certainty not identical with the melanophore active substance, the production and release of which are obvious from stage 33/34 judging from the melanophore activities (Etkin 1941; Nyholm 1969). It seems more likely that MSH is that melanophore active substance. That MSH is not demonstrable with the immunofluorescence technique until stage 37/38, may be explained in the following way: During the period of development in which the MSH-release controlling mechanism is still not developed, i.e. before about stage 40 (Nyholm 1969, 1972; Terlou et al. 1973; this paper chapter IV) the MSH produced, is released at a maximal rate, leaving minimal amounts of stored MSH. In this situation the melanophores, which react on the released MSH, are consequently the most sensitive indicator of MSH production. The immunohistological technique, which demonstrates stored MSH, is not applicable to prove MSH-production until the synthesis of the hormone exceeds the release, so that MSH is retained in the cells in demonstrable amounts. This would be the case from stage 37/38.

The idea that the rate of the MSH-release is high in relation to its synthesis during the developmental stages preceding that in which the MSH-release inhibiting mechanism gets functional, is supported by the fact that the intensity of the immunofluorescence always greatly increased as soon as this mechanism is operating. Thus during stage 39 the fluorescence intensity is still low while already during stage 40 it is always raised (figures 2a and 3a).

The increase in fluorescence intensity which occurs between stages 39 and 40 seems equally obvious whether the tadpoles have been kept on a white or a black background. As far as investigated, until stage 46, the intensity of the fluorescence remains at the same level, as estimated subjectively, in tadpoles of equal age, whether they are kept on a white or a black background. As the quantitative resolution of the immunohistological technique is unknown, this study is, however, not considered to contribute to the discussion on the effect of different backgrounds upon the MSH-content of the pars intermedia.

A hypothesis concerning the derivation, in mammals and elasmobranchs, of α-MSH and β-MSH from ACTH and β-LPH (via γ-LPH), respectively, has been put forward by Scott et al. (1973) and Lowry & Scott (1975). This idea may be supported by the finding of Whur & Weatherhead (1971) that the rate of incorporation of [^{3}H] leucine into the pars intermedia of adult Xenopus laevis changed when the frogs were transferred from a white to a black background or vice versa. This reflects a change in the rate of synthesis of a substance(s) which, however, was not specified. Leucine is lacking in the known MSH peptides, with the exception of dogfish (Squalus acanthias) β-MSH (Bennett et al. 1974) (the amphibian MSH:s are not chemically characterized), but is present in the ACTH and LPH peptides (Chrétien & Li 1967). These findings, therefore, may indicate a relation between the ACTH and/or LPH synthesis and the background, which could fit with these peptides being MSH precursors.

The failure by Hopkins (1975) to demonstrate synthesis of any [^{3}H] leucine labelled peptides in the pars intermedia of adult Xenopus seems puzzling, in the view of the clear-cut results of Whur & Weatherhead (op. cit.) in the same species.

In the present study, no sign of ACTH production in Xenopus tadpoles appeared even in the oldest of the developmental stages examined. Since α-MSH then since long was demonstrable, this study gives no support to the hypothesis that ACTH is a precursor of α-MSH in amphibians.

Doerr-Schott & Dubois (1973) give too few stages to be able to demonstrate the time relations of the earliest presence of ACTH and α-MSH in Rana temporaria tadpoles. In the foetal rat, studied with the immunohistological technique, ACTH and α-MSH appeared synchronously in the pars intermedia (Dupouy & Dubois 1975).

The immunohistological technique makes it possible to follow, in a direct manner, what happens to the MSH-producing cells in the process of cellular reorganization which occurs in the adenohypophysial rudiment during the stage 37/38 to 39 (cf. this paper chapter I). This reorganization leads to a shift of the MSH-cells from a strandlike arrangement at stage 37/38 into a cluster, concentrated in the most dorso-caudal part of the hypophysis during stage 39. MSH-producing cells in immunohistological studies have been found to be strictly bound to the pars intermedia in larval and adult amphibians (Doerr-Schott & Dubois 1972, 1973; Doerr-Schott 1974). It seems, therefore, very reasonable to consider the cluster of MSH cells as a well defined pars intermedia, even though its histological boundary against the pars distalis is not yet formed. This does not occur until stage 47 (Kerr 1966).

In the pars intermedia of stages 39 to as far as studied, the MSH-producing cells are centrally located, peripherally surrounded by non-fluorescent cells (figures 2a to 5a). Within the MSH-producing cell mass there are frequently seen regions where the MSH-immunofluorescence seems more intense than generally in the cytoplasm. With general histology these regions are found to coincide with those "spaces" which occur between the cell-bodies of the pars intermedia cells and which give this part of the hypophysis its rather loose cellular arrangement. The ultra-structure of the "spaces" reveals a heavy concentration of cell processes containing secretory granules (for further discussion, see chapter III).

The cellular organization of the pars intermedia deviates from that of the pars distalis in a manner which gets more accentuated during the development from stage 39 and further

on. In contrast to the seemingly random distribution of the pars intermedia cells those of the pars distalis become arranged in a strongly regular manner into strands which are oriented against the "hilar" region of the adenohypophysial rudiment (cf. chapter I).

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Figures 1 - 5

Immunofluorescence in hypophysial rudiments of Xenopus laevis tadpoles after treatment with anti -β- MSH antibodies. (The tadpoles were kept on a black background until the last 30 min. when they were transferred to a white.)

Figure 1a. Developmental stage 37/38. In the elongate hypophysial rudiment the MSH-producing cells are arranged in a strandlike manner in the caudal half.

Figure 2a. Stage 39. The MSH cells are now grouped in the definite position of the pars intermedia.

Figure 3a. Stage 40. The intensity of the fluorescence is increased. (The figures 2a and 3a are identically treated in the photographic procedures.)

Figure 4a. Stage 43.

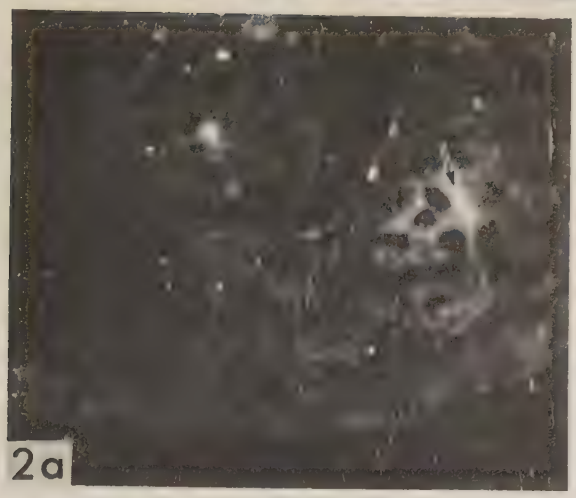
Figure 5a. Stage 46.

Figures 1b - 5b. Camera lucida drawings of the same sections as shown in figures 1a - 5a. Only the cell nuclei are indicated in the hypophysial rudiment. The dotted nuclei correspond to those of the MSH cells in the a-figures.

Note the differences of the cellular arrangemen in the pars intermedia and the pars distalis, which become successively more obvious.

V - Third ventricle; I - Infundibular floor. The arrows indicate the position of strongly fluorescent areas between the cell-bodies of the MSH-cells.


1a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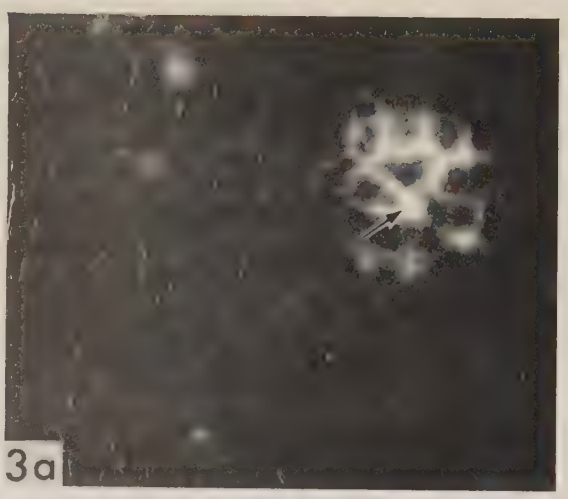
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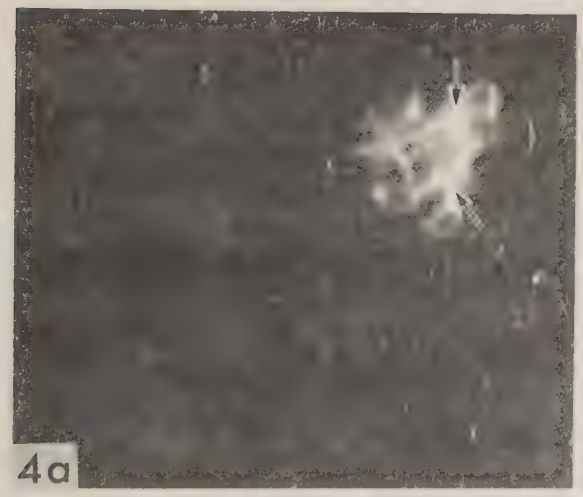
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Chapter III

AN ULTRASTRUCTURAL STUDY OF ACTIVITY AND CONTROL OF THE MSH-CELLS IN EARLY *XENOPUS LAEVIS* TADPOLES

ABSTRACT

The development of the secretory function of the MSH-cells and its control mechanism, have been studied in *Xenopus laevis* tadpoles during the developmental stages 32 to 43 (Nieuwkoop & Faber 1956).

The earliest expression of the secretory activity in the adenohypophysial rudiment is observed during stage 33/34, in a few cells with a specific localization. During the subsequent stages 35/36 and 37/38 the secretory active cell population increases to comprise the central portion along the whole rudiment. The yolk of the yolk platelets appears to be important for the process of differentiation of the MSH-cells. At least two types of yolk form the yolk platelets. One of these types is probably mainly a source of energy while the other type also forms building material and seems to contribute to the rich supply of mitochondria during the differentiation of the MSH-cells. The activity of the MSH-synthesis increases progressively to about stage 40, irrespective of the background colour. From that stage, however, only those tadpoles which are kept on a black background show indications of further increase, while in those tadpoles which are kept on a white background the regression of the RER indicates a decreased synthetic activity. From about stage 40 also the release of MSH is inhibited in white background tadpoles as evidenced by an increased storage of secretory granules in the MSH-cells. These changes of MSH-cell activity coincide with the onset of monoaminergic innervation of the pars intermedia. The monoaminergic nerve type is the only one observed within the adenohypophysial rudiment. The axons are mainly seen in the pars intermedia but do also occur in the pars distalis. Peptidergic nerve profiles are observed in the zona externa of the infundibular floor at stage 43, but do not enter the adenohypophysis.

INTRODUCTION

The ultrastructure of the adenohypophysial rudiment of larval amphibians has been the subject of only a few investigations (Dent & Gupta 1967, Triturus v. viridescens; Smoller 1966, Hyla regilla; Pehlemann 1967, Xenopus laevis, Doerr-Schott 1968, Rana temporaria; Mira-Moser 1972, Bufo bufo). These studies treat the MSH-cells and the pars intermedia only superficially and generally at relatively late developmental stages.

The early larval stages, which comprise the differentiation of the MSH-cells (Kleinholtz 1940; Drager & Blount 1941; Etkin 1941; Hegre 1946; Pehlemann 1962, 1965; Nyholm 1969) as well as the neuro-endocrine system which controls the activity of the MSH-cells (Nyholm 1969, 1972; Terlou et al. 1972, 1973), have largely been overlooked. A reason for this probably is that the localization of the MSH-cells in the adenohypophysial rudiment has been uncertain during the early stages, when the histological boundary of the pars intermedia is still not formed. This problem is now mainly solved, however, as regards the Xenopus laevis tadpoles by the immunohistological study which is presented in Chapter II. This forms the foundation for the present study.

MATERIAL AND METHODS

Tadpoles of Xenopus laevis (Daudin) were obtained from adults in which spawning was induced by injection of HCG[x)] into the dorsal lymph sac. The females and males were given about 600 I.U. and 300 I.U. respectively, as a single dose. (Henriques 1964).

The tadpoles were kept in room temperature in plastic dishes which were painted to provide a white or a black background. The backgrounds were illuminated by a 60 W lamp placed about 60 cm above the dishes.

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x) Gonadex, Leo, Sweden

The total numbers of specimens investigated of different developmental stages (according to Nieuwkoop & Faber 1956) were as shown in the table below.

Table.

Stage no	32	33/34	35/36	37/38	39	39/40	40	41	42	43
Specimens	4	3	4	11	9	13	9	4	8	4[a)]
Numbers of broods	2	1	2	3	3	4	3	3	2	2

[a)] Moreover 3 tadpoles which abnormally still could not adapt their melanophores to the background.

The heads of the tadpoles - at stages 42 and 43 omitting the lower jaw - were fixed in 2.5 % glutaraldehyde and 1 % OsO_4 according to Mercer & Birbeck (1966), dehydrated in ethanol and embedded in Vestopal W or Epon. Sagittal sectioning was carried out on a LKB Ultrotome. The sections were stained with saturated uranyl acetate in destilled water, alone or combined with lead citrate (Vanable & Coggeshall 1965) and examined in a Philips EM 300 or a Zeiss EM 10.

RESULTS

Ultrastructural indications of functional differentiation of the MSH-cells

Observations on the development of the rough endoplasmic reticulum, Golgi apparatus and secretory granules.

It is the current opinion that the synthesis of polypeptide hormones is bound to the rough endoplasmic reticulum (RER). The hormones are then segregated from the RER and transferred to the Golgi complex where they are accumulated and delivered into the cytoplasm as membrane bound primary secretory granules (Bargmann 1971; Farquhar 1971; Pehlemann 1974). There is also good evidence that the development of

these organelles in the cells reflects the intensity of the secretory activity, i.a. in Xenopus MSH-cells (i.a. Weatherhead and Whur 1972).

The number and distribution of the secretory granules in the cytoplasm of the MSH-cells are functions of the rates of synthesis and release of the hormone. Thus Hopkins (1970a) observed that in the MSH-cells of adult Xenopus laevis, during conditions of low release rate (white background), large numbers of secretory granules were accumulated and stored in the cytoplasm. At the state of high release the stores were gradually emptied and after one week, secretory granules could only be observed surrounding the Golgi complex - although the rate of synthesis was obviously high.

Stage 32 (Fig. 1). The cells of the hypophysial rudiment invariably contain very high numbers of free ribosomes. Also polysomes frequently occur.

The RER is very poorly developed as are the Golgi complexes. The latter are represented by a few flat cisternae which are surrounded by a few Golgi vesicles. Indications of secretory activity - dense material in the Golgi cisternae or secretory granules - are not observed in cells of the hypophysial rudiment.

Stage 33/34 (Fig. 2). In two out of three investigated tadpoles secretory activity could be detected by the presence of a few secretory granules in two and three hypophysial cells, respectively. In both tadpoles the granules occurred in adjacent cells located in a mid-dorsal position of median sagittal sections.

The RER, though poorly represented, is generally more developed than in the earlier stage. The cytoplasm is crowded with ribosomes as before. Frequently the cells contain more than one Golgi complex, all with flat cisternae.

Stage 35/36 (Fig. 3) and 37/38 (Fig. 4). Secretory cells, characterized in the same way as in stage 33/34, were only observed in the median region of the hypophysial rudiment

at stage 35/36. The region of their occurrance is however somewhat extended in the frontal and caudal directions. At stage 37/38, cells containing secretory granules occur along the whole length of the rudiment. In the caudal half of the rudiment, the secretory cells (MSH-cells) occur with a marked concentration to the central parts (cf. Chapter II).

The MSH-cells contain only a few secretory granules which mainly occur in the Golgi region. An increase in the number of RER-profiles is observed in the secretory cells during these stages. The cisternae of the Golgi complexes in these cells are somewhat dilated. In addition there frequently appear several Golgi complexes in each cell. These may be situated rather near one another, forming a concentrated Golgi region, or they may be more separate.

Stage 39 (Fig. 5). From stage 39 the MSH-cells are assembled into a well-defined pars intermedia in the dorso-caudal part of the hypophysial rudiment (cf. Chapter II).

The secretory granules of the MSH-cells occur in higher numbers than earlier. In the cell bodies they are heavily concentrated to the Golgi region, but granules are also observed in small numbers in processes of MSH-cells. The RER is still more extensive than at stage 37/38, while the Golgi complexes have characteristics similar to that stage. Comparison of the tadpoles kept on a white or a black background did not reveal any influences of the background colour on the structure or extent of these organelles in the MSH-cells.

Stage 40 (Figs 6, 7). The number of secretory granules in the MSH-cells seems generally to be higher than at stage 39. The localization in the Golgi region and cell processes is similar to stage 39. The RER is generally more prominent than earlier. The cisternae of that system are often organized into several parallel rows.

The Golgi complexes show characteristics similar to stage 37/38 and 39; the cisternae are somewhat dilated. The ultrastructure of the MSH-cells seems not to be influenced by the background history of the tadpoles.

Stage 41, stage 42 (Figs. 8,9) and stage 43. At these developmental stages the ultrastructure of the MSH-cells is distinctly correlated to the background history of the tadpoles. In those which were kept on a black background the MSH-cells are rather similar to those of stage 40. Thus, the RER is prominent and the secretory granules are far more numerous in the Golgi region than elsewhere in the cytoplasm of the cell bodies, where they occur in low numbers. In the processes of MSH-cells, however, secretory granules are rather frequent.

In those tadpoles which were maintained on a white background, the RER of the MSH-cells is only moderately represented. Still, the number of secretory granules is higher than in the black background tadpoles; granules occur to an increased extent in the cytoplasm outside the Golgi region, and are quite numerous in the MSH-cell processes (Fig. 10). The Golgi complexes seem equally developed in tadpoles with different background histories at stage 41 and are similar to those of stage 40. At the stages 42 and 43 the Golgi cisternae are relatively flat in white background tadpoles.

General observations. The numbers of secretory granules in the MSH-cells of *Xenopus* tadpoles, at all the stages studied, are considerably lower than in the corresponding cells of adult frogs.

All secretory granules of these cells, as far as observed, are of the so-called primary (immature) type, i.e. they are of equal size and exhibit an electron dense core surrounded by a transparent zone inside the limiting membrane (Pehlemann, 1974).

Observations on microtubules and microfilaments

Perryman (1973) observed that the frequency of microtubules and microfilaments changed when the secretory activity of the MSH-cells varied, in adult *Rana pipens*. These organelles were much more frequent in the more active MSH-cells (in black background-adapted frogs) than in the less active

(white background). Generally the microtubules are believed to support the motility of the cell itself, and the intracellular movements of organelles (review by Bencke 1974).

In the MSH-cells of Xenopus tadpoles in the present study microtubules and microfilaments are very often observed in the cell bodies as well as in the processes. In the cell processes these organelles appear to be much more frequent after the tadpoles have reached about stage 40 (Figs 11, 12) than at earlier stages. The present material does however not reveal any differences in the frequency of microtubules and microfilaments in tadpoles with different background histories.

Observations on yolk and yolk utilization

It is well established that the yolk material in the cells of the amphibian embryo constitutes a reservoir which is utilized during the cell differentiation as a source of energy and building material (cf. Karasaki 1963).

The present study reveals that the yolk utilization, as reflected in the degree of yolk platelet disintegration, is very extensive in the hypophysial rudiment of Xenopus tadpoles during the earliest embryonal stages - 33/34 and 35/36. The majority of the yolk platelets seems to be utilized during these stages.

A subjective assessment of the distribution of the yolk platelets indicates that this is very similar throughout the hypophysial rudiment during the earlier stages. However, from about stage 39 it seems obvious that the cells of the pars intermedia generally contain less yolk than do the pars distalis cells. Normally the yolk platelets of the pars intermedia seem to be fully disintegrated at about stage 40. After some additional stages, at about stage 42, also the pars distalis normally is depleted of its yolk.

The yolk of the yolk platelets has a special crystalline structure which is identical in several amphibian species, including Xenopus laevis (Ward 1962; Karasaki 1963; Wallace 1963; Lanzavecchia 1965). The intact yolk platelets are surrounded by a limiting membrane.

The present study reveals that the structure of the yolk platelets of the hypophysial cells of Xenopus is variable. In the dominating number of cases the crystalline pattern is uniform throughout the platelet, indicating that they are homogeneous (Fig. 13). In the remaining fraction of yolk platelets the direction of the crystalline pattern shows regional variations, which indicate that the platelets in these cases are composed of several subunits (Fig. 15). It is uncertain whether the subunits are surrounded each by a limiting membrane.

In different Rana-species there occurs intramitochondrial yolk (Karasaki 1963), which from the pattern of the crystalline lattice and the course of disintegration is interpreted to be different from the yolk of the platelets (Lanzavecchia 1965).

As far as has been observed the "homogeneous" yolk platelets, without exceptions, are disintegrated through a progressive digestion from the periphery (Fig. 14). It is uncertain whether a limiting membrane may closely surround the yolk body during the disintegration.

In the "composite" platelets the yolk digestion as a rule goes on from the periphery of each crystal. The process of disintegration of these yolk platelets is coupled with the formation of various material which may be characteristic for different yolk types. The "composite" yolk platelets, with very rare exceptions, seem to be fully disintegrated before stage 37/38.

In about half the number of the "composite" platelets, membranes frequently are observed in the periphery of the yolk crystals, but very few other structures appear during the disintegration (Fig. 15).

The disintegration of the remaining "composite" yolk platelets is suggested to pass the following phases:

a. The disintegration of the yolk crystals is associated with a spectacular formation of granular material. The grains, which are of the size of ribosomes, remain when the yolk crystals are domolished, and together with the limiting membrane they form a more or less dense residual body (Fig. 16). Membranes seem to be formed only to a very limited extent during the process of yolk crystal disintegration.

b. In the residual bodies there arise rather diffusely limited patches of condensed material which are interpreted as originating from the granular matter. The patches seem to have a tendency to expand - frequently larger areas of condensed material occur which are suggestive of being formed from patches that have coalesced (Fig. 17).

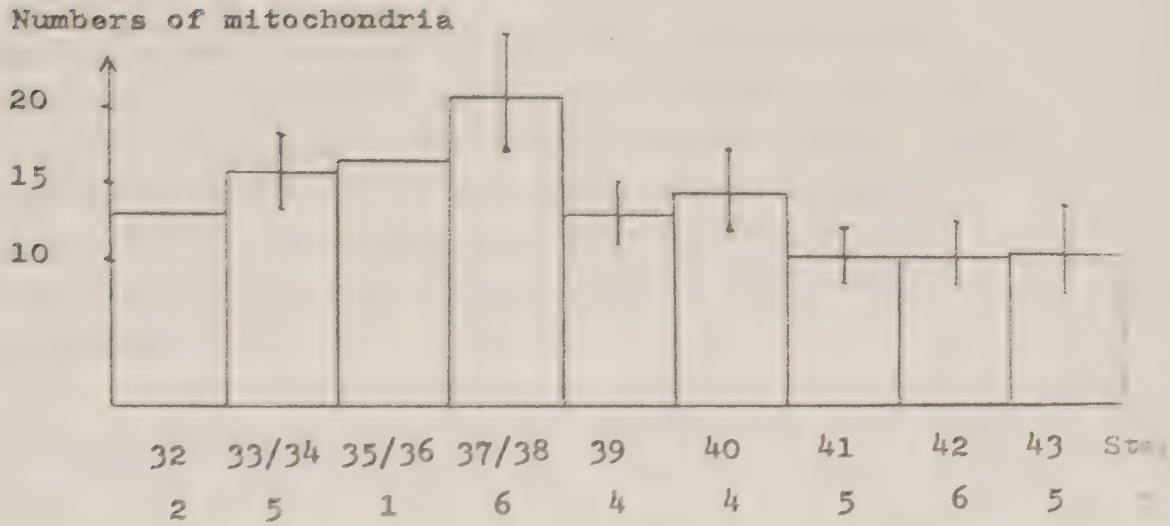
c. The further process is characterized by the formation of membraneous profiles which to a very striking extent are associated with the patches of condensed material. The formed membranes are usually double- or multilayered and describe closed figures (Fig. 14). Within residual bodies which are in a final state of disintegration, mitochondria are observed besides other membraneous structures. (See further below: "De novo mitochondrial formation".)

Observations on mitochondria

The mitochondria seem to be rather evenly distributed in the cytoplasm of the hypophysial cells up to stage 33/34. In the secretory cells of the caudal half of the hypophysial rudiment at stage 35/36, and in those cells are verified as MSH-cells at later stages, they are concentrated to the Golgi region. Mitochondria are only rarely observed in processes of the MSH-cells, and then essentially in their proximal portions.

The numbers of mitochondria in MSH-cellbodies, as revealed by counting on the electron micrographs, seem to vary during the stages studied. The highest numbers are observed at about stage 37/38. There is a progressive increase during the preceding stages, and a rapid decrease after stage 37/38 (Text-fig. 1). The decrease seems to occur independently of the colour of the background on which the tadpoles were kept.

Textfigure 1 Numbers of mitochondria as counted on electron micrographs of cell-bodies of the caudal part of the adenohypophysial rudiment at different developmental stages[x)].



x) The identity of the cells as MSH-cells is uncertain at earlier stages than 37/38.

The observations on the formation and degradation of mitochondria fit well with the indicated course of variation in their numbers. Thus profiles which are suggestive of extensive mitochondrial formation are frequently observed during stages 33/34 and 35/36. These indicate that the process occurs in two ways:

a. De novo formation from disintegrating yolk material.

A minority of the yolk platelets are disintegrated under the formation of a membrane-limited granular body in which patches of condensed matter are formed (cf. above, and Fig. 17). Very frequently these patches are associated with membranes. The membranes, which strikingly often are double-layered, adopt a ring-structure which in most cases enclose a part of, or a complete patch of condensed material.

During the further process the residual bodies of the yolk platelets gradually disintegrate, possibly with some partitioning into smaller units (cf. Karasaki 1963). Within those residual bodies which are interpreted as being an advanced stage of disintegration, mitochondria may be observed (Figs 18 a-d) as well as several membraneous configurations.

The internal structure of these mitochondria appears less developed than in those mitochondria which are free in the surrounding cytoplasm, i.e. as regards the cristae system. In one mitochondrion within a residual body, vesicular structures were observed (Fig. 18 a) which closely resemble those which Schjeide et al. (1964) and Schjeide (1970) interpreted as rudiments of cristae in "mitochondrial buds" of early chick embryos.

This may imply that those mitochondria that remain within the residual bodies are not fully differentiated. Matrix granules may appear in the mitochondria which are within the residual bodies. It is postulated that these mitochondria are formed within the residual bodies of the yolk platelets.

b. Division of pre-existing mitochondria

Indications of mitochondrial multiplication in this way was observed in the present material only once. Two mitochondria in the same cell, at stage 33/34, appeared to be in a state which indicated a division (Fig. 19), as described by Larsen (1970).

A decrease in the number of mitochondria occurs in the MSH-cells from about stage 39 (Text-figure 1). This fits well with the observation that structures which are suggestive of mitochondrial degradation are very rarely seen in tadpoles earlier than stage 39, but rather frequently after that stage. There is thus indication that mitochondrial degradation occurs in Xenopus tadpoles, as is also observed in several other animal species - from insects to mammals (reviewed by Tandler and Hoppel 1972, p 34). This implies that there is an engulfment of senescent of damaged organelles into autophagic vacuoles (Figs 22, 23).

From about stage 39 it was observed that a certain number of the mitochondria of the MSH-cells have become rounded and that their internal structure has become rather amorphous (Fig. 20). The significance of these structural changes, although they are striking, is not known.

Observations on lytic bodies

In the literature, secretory granules of hypophysial cells have been observed to fuse with lysosomes (Farquhar 1971, in rats; Masur and Holtzman 1969, and Doerr-Schott 1970, in amphibians). This phenomenon, which has been called "crinophagy", is interpreted as being involved in the regulation of the secretory process in the pars distalis, by elimination of temporary excess of secretory products (Farquhar 1971). In the present study, observations of dense bodies, suggestive of lysosomes, are more frequent in the oldest stages (Figs 8, 9), but no definite indications of crinophagy have been obtained.

On the basis of several studies in different mammalian tissues, Novikoff et al. (see review by Beams and Kessel 1968, p. 260-262) have proposed that lysosomes may arise from portions of smooth endoplasmic reticulum which are related to the Golgi complex - the GERL-system (Golgi Endoplasmic Reticulum Lysosomes). Hopkins (1970 b), in Xenopus, observed systems of bodies structurally similar to the GERL-system, which showed acid phosphatase activity.

In the present study, complexes with the structural characteristics of the GERL-complex are rather frequently observed in the MSH-cells at developmental stages later than about 39 (Fig. 21).

Ultrastructural indications on the innervation of the pars intermedia

It is generally agreed that the release of MSH from the amphibian pars intermedia of the hypophysis is controlled, at least partly, by direct innervation of the MSH-cells. The nerves involved in this control originate in the hypothalamus and their action is inhibitory upon the MSH release (see review by Terlou et al. 1974). The objective of the present study is to reveal the ontogenesis of the innervation of the pars intermedia in Xenopus tadpoles, at the ultrastructural level.

During the developmental stages 35/36 and 37/38 a number of axons emerge from the ventro-caudal region of the neuropile of the optic chiasma. These axons soon form a heavy bundle which gradually extends caudad in the external part of the infundibular floor. The migrating hypophysial rudiment is partly separated from the nerve bundle only by the basal lamina which covers the brain floor.

The earliest nervous profiles within the hypophysial rudiment are observed at late stage 39. Only a few axons are observed in the rudiment at the stage, however (Fig. 24). But during the following stages the nervous invasion of the hypophysial rudiment is extensive. The axons enter at several sites - from well frontal of the "hilar" region to the level of

the pars intermedia - the latter being the most common. It is not possible to determine whether all these fibres are destined to the pars intermedia, but if this were to be the case a number of axons have to pass through the pars distalis for a long distance.

The appearance of the axons breaking through the basal lamina and the layer of fibroblasts in order to enter the hypophysial rudiment may be quite striking, as demonstrated in Fig. 26.

At stage 41 axons are very frequently observed in the pars intermedia. They may form real bundles (Fig. 25), but usually they are represented by some profiles in the interstices (Fig. 8), or among the conglomerations of MSH-cell processes which occupy certain regions of the pars intermedia (cf. Chapter I).

The axons observed in the hypophysial rudiment during the developmental stages under study are interpreted as representing the same type. They are characterized by their contents of neurotubules, mitochondria, small clear vesicles (about 400 Å) and dense core vesicles (700-900 Å) (Figs 24, 25). Frequently small axonal profiles are observed which lack these organelles except for the tubules. These profiles are interpreted as intervaricose portions of the axons (cf. Fig. 24 and 25). The characteristics of the observed nerve type suggest that it is monoaminergic.

Structures which are interpreted as synaptic contacts between this nerve type and the MSH-cells are observed on cell bodies as well as on cell processes (Figs 25, 27). Glia cell processes may, as in other amphibians (e.g. Franzoni and Fasolo, 1975), accompany the nerve fibres into the pars intermedia (Fig. 25).

A different nerve type is observed in the external zone of the infundibular floor in some tadpoles at stage 43. In this case the nerves are often crowded with large granules (900-1500 Å) (Fig. 28). These observations indicate that the earliest peptidergic nerve fibres reach the hypophysial region about stage 43. They were however not observed within the hypophysial rudiment in the stages under study.

Some ultrastructural observations on the adenohypophysial rudiment of *Xenopus laevis* tadpoles with delayed capability of background adaptation.

In one brood it was observed that about 5 % of the tadpoles at stage 43 still were not able to adapt their melanophore status to a white background. This capability, however, was developed at stage 46. The remaining tadpoles in that brood demonstrated the normal pattern of background adaptation.

The ultrastructural examination of the adenohypophyses of the abnormal tadpoles revealed some features which may be of interest for the interpretation of some differentiational events in the normal adenohypophyses.

The organization of the pars intermedia cells appears to be accomplished as in normal tadpoles.

The secretory activity of the pars intermedia cells is extensive as revealed by the well developed RER (fig. 30) and Golgi complexes. The occurrence of secretory granules is limited to the Golgi region. Obviously the MSH-release is high.

A rich content of yolk material (yolk platelets and lipid globelets) is still retained in the pars distalis cells at state 43 (fig. 29). In the pars intermedia, however, the yolk is essentially consumed (fig. 30).

At stage 43, nerve bundles of normal extent occur in the external zone of the infundibular floor but no axons are observed within the adenohypophysial rudiment. This indicates that the pars intermedia is still not innervated. The axons in the infundibular floor are all interpreted as monoaminergic.

DISCUSSION

Localization of the MSH-secreting cells

Cells which are classified as secretory, from their content of secretory granules, are observed in small numbers in the hypophysial rudiment of Xenopus laevis from the developmental stage 33/34. In two out of three investigated tadpoles in which secretory granules were detected at that stage, the secretory cells, which were adjacent to one another, are observed to occur in the same region of the rudiments - mid-dorsal in median sagittal sections. At stage 35/36 secretory cells occur in the hypophysial rudiment of all the tadpoles. The region which they then occupy appears to be increased in relation to that of stage 33/34 in the frontal and caudal directions. At stage 37/38 secretory cells occur along the whole length of the rudiment. From these observations it may be concluded, with regard to the secretory function, that the hypophysial cells are asynchronously differentiated and that the differentiation start from a definite region of the rudiment.

The secretory population of the hypophysial cells is located along the central axis of the rudiment at stage 35/36 and 37/38. The cells which limit the rudiment thus are generally non-secretory. The location of the MSH-cells at stage 37/38, as immunohistologically determined (see chapter II), agrees with that of the secretory cells of the caudal half of the rudiment. This suggests that the whole secretory population of the cells in that part is constituted by MSH-cells at stage 37/38. After the reorganization of the hypophysial cells, at stage 37/38 to 39, the localization and arrangement of the MSH-cells are changed (see chapter II).

In hypophysectomized and normal Xenopus laevis tadpoles, the melanophore activity suggests that a melanophore-active substance is released from the hypophysial rudiment from about stage 33/34 (Etkin 1941; Nyholm 1969; Terlou et al. 1973). Pehlemann (1962, 1965) showed that the production of melanophore-stimulating substance is the earliest activity

of the adenohypophysial rudiment in amphibians, including Xenopus laevis. As ACTH is unlikely to be produced in the hypophysial rudiment of Xenopus at stage 33/34 (cf chapter II) it may be concluded that the secretory cells which are observed at that stage are MSH-cells.

In other amphibian species there seems to be an equally good agreement between the earliest ultrastructurally detectable secretory activity and the earliest experimentally determined occurrence of melanocyte-stimulating activity in the hypophysial rudiment (Burch 1946, Smoller 1966, and Thurmond 1967, in Hyla regilla; Pehlemann 1962, 1965, and Doerr-Schott 1968, in Rana temporaria).

The secretory activity of the MSH-cells.

The present study suggests that the differentiation of the secretory activity of the hypophysial cells is first expressed at a definite stage, 33/34. Supposedly these first secretory active cells are MSH-cells (see above).

At stage 33/34 the RER and Golgi complex are only poorly developed. During the further development of the tadpoles, these organelles progressively become more prominent in the MSH-cells, with the exception of specimens which are kept on a white background. In those specimens, from about stage 41, the RER is regressed and the cistenae of the Golgi complexes generally are flat. The characteristics of the RER and Golgi complexes suggest that the activity of MSH-synthesis progressively increases irrespective of the colour of the background, to about stage 40. The secretory granules are mainly localized to the Golgi region and, at stage 39 and 40, also in low numbers in processes of the MSH-cells. These conditions - with low storage of MSH-containing granules - suggest that the increasing synthesis is balanced by the release.

A further increase of the MSH-synthesis activity, beyond that of stage 40, seems only to occur in those tadpoles which are kept on a black background. In the tadpoles which are kept on a white background, the MSH-synthesis obviously

decreases from stage 41.

The connection between the background colour and the development of the RER and the Golgi complexes of the MSH-cells, which indicates a background dependent MSH-synthesis activity, also occurs in adult amphibians: Cohen (1967), Pehlemann (1967), Ito (1968, 1971), Imai (1969), Hopkins (1970 a,b) and Weatherhead & Whur (1972) in Xenopus laevis; Saland (1968) in Rana catesbiana and R. pipiens; Ito (1969) in R. nigromaculata; Castel (1972) in R. catesbiana and Perryman (1974) in R. pipiens. As in the Xenopus tadpoles, which are older than about stage 40, RER and Golgi complexes of the MSH-cells showed increased development as compared with the white background specimens.

These studies also reveal that the number, distribution and structural characteristics of the secretory granules of the MSH-cells are different in frogs with different background histories. With regard to the numbers and distribution of the granules, the present study agrees with the above mentioned. Thus at stages from about 41, the MSH-cells in Xenopus tadpoles kept on a black background contain relatively few secretory granules. These are located in processes of the cells, but mainly in the Golgi region of the cell bodies. In tadpoles which are kept on a white background the number of secretory granules of the MSH-cells is increased - markedly in processes of these cells but also in regions of the cell bodies outside the Golgi region. The distribution of the secretory granules in the MSH-cells of the tadpoles indicates that the intracellular transport of the granules from the site of manufacturing, preferably leads them out of the cell bodies, into processes of the cells (cf chapter II).

The observations on the number and distribution of the secretory granules suggest that in tadpoles which are kept on a black background, the obviously considerable activity of MSH-cynthesis is balanced by the MSH-release. In the white background tadpoles, on the other hand, the release is inhibited so that even the obviously decreased synthesis activity admits an increased storage of the hormone. This is in agreement with Burgers et al. (1963) and Thornton (1971),

who demonstrated that the MSH-content of the pars intermedia in adult Xenopus laevis was higher in specimens which were adapted to a white background than in those adapted to a black background.

Saland (1968) and Ito (1971) suggested that the control of MSH-synthesis is mediated by an intracellular feedback mechanism, activated by the amount of stored hormone. That model could be valid also for the control of MSH-synthesis in Xenopus laevis tadpoles, as revealed by the present study. However, this does not exclude the possibility of direct nervous control of the MSH-synthesis. Perryman (1974) in Rana pipiens suggested a negative feedback action by circulating MSH upon the release and further the synthesis of MSH. The results of experiments, in vitro, on neuro-intermediate lobes of Bufo arenarum, by Iturriza (1973) may be interpreted in a similar way. These last-mentioned studies thus imply that, when the amount of circulating MSH is high, the release and/or the synthesis of the MSH is inhibited. Such a mechanism, however, seems not to be consistent with the ultrastructural evidences obtained in amphibians, concerning the development of the RER and Golgi complexes in the process of normal background adaptation. These organelles attain an extensive development, which is retained as long as the frogs are kept on a black background - when the amount of circulating MSH is the highest.

With regard to the structural characteristics of the secretory granules of the MSH-cells, in the Xenopus tadpoles comprised in the present study, all appear to be so-called "primary granules". [^{3}H] -glycine incorporation experiments show that these granules contain MSH (Hopkins 1972). In the MSH-cells of adult amphibians and Rana temporaria tadpoles of prometamorphic stage (Doerr-Schott 1968), a fibrous type of granules occurs besides the "primary" granules. The numbers of the fibrous granules are significantly reduced in frogs which are transferred from a white to a black background. However, the relation, of these granules, to MSH-secretion is unclear (Weatherhead & Whur 1972, Hopkins 1972, and Kikuyama & Yasumasu 1972).

Microtubules and microfilaments.

The microtubules and microfilaments are generally believed to support the motility of the cell itself and the intracellular movements of organelles (see review by Bencke 1974).

The very rich occurrence of microtubules and microfilaments in the processes of MSH-cells from about stage 40 may support the transfer of secretory granules into these processes. As mentioned above, the secretory granules at these stages are preferably transported out of the cell bodies and into the processes, where they occur in relatively high amounts.

The microtubules and the microfilaments in the MSH-cells seem to be equally frequent whether the tadpoles are kept on a white or a black background. These observations may not agree with that of Perryman (1973). She found that stimulated secretory cells of the pars intermedia of adult Rana pipiens had a higher content of these organelles than had unstimulated cells. The studies may not be comparable, however, as the Xenopus tadpoles of the present study had been capable of MSH-cell control, according to the background colour, for less than about 24 hours, while the R. pipiens studied by Perryman (op cit.) were background adapted for 14 days.

Yolk and yolk utilization.

The amphibian yolk is composed of two components - a phosphoprotein (amphibian phosvitin) and a lipoprotein (amphibian lipovitellin) which together form a crystalline body (Wallace 1963). The yolk in amphibian oocytes and embryos appears as yolk platelets. The crystalline material of these platelets is found to have identical crystal lattices in several amphibian species, including Xenopus laevis (see Lanzavecchia 1965). In several Rana-species it has been observed that yolk crystals, besides yolk platelets, also may occur as intramitochondrial yolk in oocytes and embryos (see Massover 1971 a). The ultrastructure of the crystalline lattices, together with the cours of the

demolition, suggest that the yolk platelets and the intramitochondrial yolk represent two different forms. The differences of the crystalline lattices may reflect different portions between the phosvitin and lipovitelline components (Lanzavecchia 1965).

The present study reveals that in the adenohypophysial rudiment of Xenopus laevis tadpoles the yolk is present as yolk platelets and lipid globelets, which are prominent structures in all the cells of the rudiment at the earliest developmental stages studied. The ultrastructure of the yolk platelets shows that these are composed in various ways. In the majority of the platelets the crystalline pattern is uniform throughout. This character is, as far as can be estimated, in agreement with the yolk platelets of other anurans (e.g. Karasaki 1963). Frequently, however, yolk platelets in the Xenopus tadpoles are characterized by variously directed crystalline patterns in different regions of the platelets, as though these were composed of several smaller crystals.

The utilization of the yolk material seems to be very extensive in the hypophysial cells during the stages 33/34 and 35/36. During this period most of the yolk crystals are distintegrated.

From about stage 39 it becomes obvious that the cells of the pars intermedia contain less yolk than do the pars distalis cells. The yolk platelets are also fully disintegrated at a somewhat earlier stage in the pars intermedia than in the pars distalis. This indicates that the rate of yolk utilization is higher in the former lobe.

The process of disintegration proceeds through a progressive digestion from the perifery of the yolk crystals. In the case of the "composite" yolk platelets this implies that the individual crystals aré digested simultaneously. This should mean that the process of digestion covers a far larger crystal-surface in the "composite" platelets than in those which are constituted by only one large crystal, and lead to a faster disintegration of the former. This agrees with the observation that "composite" yolk platelets,

as a rule, are extinct at stages beyond 35/36, while remnants of "unitary" platelets occur considerably later. This study, however, does not exclude the possibility that other factors, as well, promote the relatively faster disintegration of the "composite" platelets.

During the disintegration of the "composite" yolk platelets, membraneous structures are frequently observed in association with the crystals, sometimes more or less surrounding them. It has not been possible to establish whether these membranes are newly formed during the process of disintegration or whether they represent remnants of limiting membranes of the individual yolk crystals. No structures are observed, however, which could indicate the presence of such membranes in the intact yolk platelets.

One set of the "composite" yolk platelets demonstrates characteristics during the disintegration which have similarities with the "granular" type of yolk disintegration (Karasaki 1963). The disintegration of these platelets in Xenopus tadpoles is associated with an extensive formation of granular material, among which membranes are formed. These membranes are generally organized into double or multi-layered structures which are formed in association with patches of amorphous matter, interpreted to originate from the granular material. It is suggested that mitochondria may be the end-product in a process of further differentiation of these membraneous formations (see further p. III:26). The course of yolk disintegration as revealed by the present study is summarized on fig. 31.

The present study reveals, as in Rana-species (Lanzavecchia 1965), that the yolk occurs in at least two different types in Xenopus laevis. Moreover, one of the yolk types, also in the latter species is associated with mitochondria, even though this association is differently expressed in the two frog genera.

The yolk material constitutes a reservoir which is utilized during the cell differentiation as a source of energy and building material (cf Karasaki 1963). Since it has been

shown that amphibian yolk platelets contain DNA (Hanocq et al. 1972) as well as RNA (Kelly et al. 1970, cited by Hanocq et al. op cit.), it is also believed that the yolk platelets in a more active way may contribute to the development. In this connection one might remind of the hypothesis that amphibian yolk may be associated with embryonic induction (cf Flickinger 1956).

In Xenopus, the present study gives some evidence for the occurrence of these functions of the yolk platelets. The "unitary" platelets are digested without formation of visible structures and therefore might be interpreted mainly as energy sources.

One phase of the process of the digestion of the "composite" platelets is associated with formation of membranes which are suggested to build up the mitochondria which occur in residual bodies of the yolk platelets.

An indication of possible inductive function of the yolk platelets arises from the innervation of the adenohypophysial rudiment. In normal tadpoles, axons are first observed within the rudiment at advanced stage 39, at which stage the yolk content of the rudiment has become very low, especially in the pars intermedia. In tadpoles which abnormally had not acquired the capability of colour adaptation to a white background at stage 43, it was not possible to find any axons in the adenohypophysial rudiment despite a seemingly normal number of fibres in the external zone of the infundibular floor. The hypophysial rudiments of these tadpoles also differed from the normals by the presence of high amounts of yolky material, especially in the pars distalis. At stage 46, also the abnormal tadpoles had acquired the capability of normal colour adaptation, which indicates that the pars intermedia then was innervated. As no specimens of that stage were secured, however, a possible correlation between the hypophysial nerve supply and the amount of yolk could not be fully elucidated.

Mitochondria

The requirements for mitochondria are high in cells which are undergoing differentiation (Schjeide 1970). These requirements are provided for by an increased total mitochondrial mass in the differentiating cells as observed in several animal species. The ways by which the increase is reached vary: It may be due to an increase in the number of mitochondria or in their individual size, or both (review by Afzelius 1973).

In Xenopus tadpoles, counts of mitochondria from electron micrographs of cells classified as MSH-cells indicate that a maximal number is reached at about stage 37/38. The trend of increase during the stages 33/34 and 35/36 and decrease later than stage 37/38 is supported by observations which indicate mitochondrial formation and degradation respectively.

The trend of the variations of the mitochondrial numbers in the MSH-cells does not follow that of the secretory activity. It may be concluded that an extensive mitochondrial supply is needed for the early differentiation of the secretory function of the MSH-cells, but not for maintaining the furhter secretory activity.

The problem of mitochondrial formation is a subject of much controversy. Different modes of formation of this organelle have been proposed: a/ Division of pre-existing mitochondria, b/ De novo formation, c/ transformation from non-mitochondrial membrane systems (Schjeide 1970; Tandler & Hoppel 1972).

In the Xenopus tadpoles structures are observed which suggest mitochondrial formation during the stages 33/34 and 35/36. This process seems to go on in two ways: a/Division of pre-existing mitochondria and b/ de novo synthesis from yolk material (Fig. 31).

That mitochondria multiplicate by the mode of division is generally accepted and is consistent with these organelles having their own DNA, ribosomes and the potential to

synthesize protein (cf Afzelius 1973). Mitochondrial division is observed in several animal species and is indicated also in amphibians - in the embryo of a Triturus (Karasaki 1959, as cited by Afzelius op. cit.).

De novo formation of mitochondria has been proposed to occur in a variety of ways in different organisms (Schjeide 1970). The origin of mitochondria from yolk material is proposed for embryonic tissues, which are rich in yolk, such as chick blastoderm (Emanuelsson 1968) and amphibians (Lanzavecchia & Le Coultre 1958; Sung 1961).

In amphibians, associations between mitochondria and yolk crystals are apparently only described from Rana-species. The occurrence of yolk crystals within mitochondria, in the oocytes and early embryos of Rana-frogs have inspired workers to propose that these crystals are the initial crystalline element of the yolk platelets (Ward 1962) and conversly, that mitochondria may originate from the yolk platelets (Lanzavecchia & Le Coultre 1958; Sung 1961). In a later work by Lanzavecchia (1965) these proposals are doubted since it was found that the intramitochondrial yolk demonstrated a specific crystalline lattice and also was demolished in a way different from the yolk platelets. More recently, however, indications on expulsion from mitochondria of the intramitochondrially formed yolk crystals are described in Rana catesbiana oocytes (Massover 1971 b). This author also mentions that observations of intramitochondrially formed yolk crystals outside mitochondria were relatively infrequent. These findings may support the possibility of mitochondrial formation from yolk platelets as proposed by Lanzavecchia & Le Coultre and Sung (op cit.).

The present study appears to give the first observations of a close association between yolk platelets and mitochondria in amphibians except for Rana-species. As in Rana, only a minority of the yolk platelets are involved and these platelets demonstrate specific characteristics, but otherwise the yolk - mitochondrion association expresses great structural differences in the two amphibian genera.

The postulate that mitochondria in the adenohypophysial rudiment of Xenopus laevis are formed within yolk platelets during developmental stages comprising cell differentiation, rests on structural observations, the validity of which will be discussed: The bodies in which the mitochondria are observed, can with reasonable certainty be considered as formed from yolk platelets, during the process of disintegration, as several transitional stages are observed.

Locke & Collins (1965) demonstrated in the fat body of an insect that the engulfment of cytoplasmic portions, including mitochondria, into bodies which were interpreted as autophagic vacuoles, occurred during a well defined period before pupation. Such vacuoles were observed eventually to be transformed into "protein granules" in which crystallization was observed. In this way bodies were formed which were structurally similar to partly disintegrated yolk platelets as observed in the Xenopus tadpoles. The interpretation that bodies with enclosed mitochondria refer to early states of mitochondrial degradation in a process of yolk formation is contradicted by the fact that the hypophysial cells are in a state of differentiation and marked yolk utilization. It may thus be concluded that the bodies in which the mitochondria are found represent residual bodies in states of final yolk platelet disintegration.

The possibility remains that the mitochondria are not formed within the residual bodies, but have migrated into them. However, it seems reasonable to believe that the mitochondria which are found within the residual bodies of yolk platelets are in a state of formation as they show structural characteristics which are suggestive of progressive morphogenetic differentiation (i.e. as regards the cristae). Moreover, if mitochondria frequently penetrate into residual bodies it seems probable that some would be observed during the state of penetration - this is not the case, however.

Structural evidence thus is suggestive of mitochondrial origin from yolk platelets in Xenopus laevis. Although not within the scope of the present study the following questions now may arise: Why does only a minority of the membraneous formations in the residual bodies of the yolk platelets organize intomitochondrial configuration? How does mitochondrial formation agree with the lytic conditions of the yolk platelet during the disintegration?

The differences between the formation of the membraneous configurations in common and the configuration of the mitochondria may be related to the presence of a specific organizer for the mitochondrial formation. As such the specific mitochondrial DNA molecule (Borst 1972) may be suggested. So far, however, it apparently has not been possible to demonstrate the occurrence of mitochondrial DNA in amphibian yolk platelets, but well of nuclear DNA, in different species studied, including Xenopus laevis (Hanocq et al. 1972). As the present study reveals that mitochondria are found in only relatively few yolk platelets and these obviously each contain only a few mitochondria it may be that only still more careful searches could reveal the existence of mitochondrial DNA in yolk platelets.

The lysis of the yolk in the hypophysial rudiment of Xenopus laevis generally directly leads to the total disappearance of the yolk platelets without formation of structures bound to it. With regard to the type of yolk platelets in which mitochondria are found the disintegration occurs in different steps. The membrane formation in these platelets, essentially, is not initiated among the formed granular material before all yolk crystals are disintegrated. Perhaps the enzymatic conditions have then changed to be more favourable for membrane formation. Only enzymatic investigations, however, can show the actual lytic conditions.

Lytic bodies

On the basis of several studies in different mammalian tissues it has been proposed that primary lysosomes arise from portions of smooth endoplasmic reticulum which are related to the Golgi complex - the GERL-system (Novikoff et al. 1964, 1966 as reviewed by Beams & Kessel 1968).

In the Golgi region of MSH-cells in adult Xenopus laevis Hopkins (1970 b) observed reticular systems which were structurally similar to the GERL-system as described in mammalian tissues. He also demonstrated acid phosphatase activity in these systems.

In the MSH-cells of the Xenopus laevis tadpoles, structures with the characteristics of the GERL-complex are rather frequently observed at developmental stages later than about 39. From that stage also observations of autophagic vacuoles in the MSH-cells are more frequent.

It is concluded that the formation of lysosomes in the MSH-cells is more extensive after about stage 39 than before. The appearance of increased numbers of lytic bodies might imply that the MSH-cells from that stage reach a new physiological state.

Innervation

The present study strongly indicates that all the axons within the pars intermedia of the hypophysial rudiment of Xenopus laevis tadpoles, to stage 43, represent the same nerve type - the monoaminergic type, which is of central nervous origin. This is in agreement with ultrastructural observations in larvae of other amphibian species (Dent & Gupta 1967, Triturus v. viridescens; Smoller 1966, Hyla regilla; Doerr-Schott 1968, Rana temporaria; Mira-Moser 1972, Bufo bufo; Pehlemann 1967, Xenopus laevis).

In the present study the earliest observation of axons within the pars intermedia are made at advanced stage 39. At that stage the adenohypophysial rudiment has still not finished its "migration". In Hyla regilla axons penetrate into the pars intermedia at about the corresponding stage of development - during the final period of the adenohypophysial "migration" (Smoller 1966). In Rana temporaria, however, the innervation of the pars intermedia obviously starts at a more advanced stage. Doerr-Schott (1968) does not give the exact scheme for the onset of the innervation in the Rana temporaria tadpoles - only that axons occur in the pars intermedia at a stage of premetamorphosis.

Several ultrastructural studies have considered the innervation of the pars intermedia in adult amphibians: Doerr-Schott & Follenius (1972). Triturus alpestris; Franzoni & Fasolo (1975). T. cristatus; Iturriza (1964), Bufo arenarum; Saland (1968), Rana pipiens and R. catesbiana; Nakai & Gorbman (1969), R. pipiens; Castel (1972), R. catesbiana; Doerr-Schott & Follenius (1970), R. esculenta; Ito (1969, 1971), Pehlemann (1967), Imai (1969) and Hopkins (1970 a, 1971), Xenopus laevis. All these studies agree that the pars intermedia is innervated by nerves with the structural characteristics of the monoaminergic type - small clear vesicles (200 - 500 Å) and dense core vesicles which are less than 1000 Å. The identity of these nerves as monoaminergic is verified by electron microscopy autoradiography in Rana esculenta (Doerr-Schott & Follenius 1970) and Xenopus laevis (Hopkins 1971). As in the Xenopus laevis tadpoles of the present study, several of the above mentioned studies reveal synaptic contacts between the monoaminergic nerves and the secretory cells of the pars intermedia.

In the adult amphibian a second nerve type is generally observed among the pars intermedia cells. This has the characteristics of the peptidergic type - with electron dense granules which measure 1000 - 2000 Å. Synaptic contact between the peptidergic nerves and secretory pars intermedia cells are observed in Rana pipiens (Nakai & Gorbman 1969). These authors also observed synaptic contacts of peptidergic

as well as monoaminergic neurons on the same secretory pars intermedia cell.

Doerr-Schott & Follenius (1970) in the pars intermedia of Rana esculenta observed axonal profiles which might represent a third nerve type. These profiles were lacking electron dense granules and did not demonstrate incorporation of tritiated noradrenalin as did adjacent monoaminergic nerves.

The ultrastructural studies of adult amphibians thus reveal a rather complex pattern of the innervation of the pars intermedia, as compared with that of the amphibian larvae.

The functional significance of the different nerve types in the pars intermedia of adult amphibians have been the subject of several studies but is still a matter of controversy. Much evidence from morphological, pharmacological and physiological studies has accumulated, however, which suggest association of the monoaminergic neurons with the mechanism of MSH-release inhibition (see review by Terlou et al. 1974; Lim Shin Chong 1973).

The present study reveals that the onset of the monoaminergic innervation of the pars intermedia in Xenopus laevis tadpoles, at advanced stage 39, coincides with the capability of the tadpoles to adapt their melanophore states to white and black backgrounds (cf chapter IV). The significance of the innervation for the capability of background adaptation is supported by those tadpoles which abnormally, at stage 43 still lacked nerves in the adenohypophysial rudiment and also the capability to adapt to the background colour. The MSH-release inhibiting function of the monoaminergic nerves in the Xenopus laevis tadpoles is indicated by the storage of secretory granules which occur in the MSH-cells in those tadpoles which are adapted to a white background (see above). The present study thus supports a monoaminergic nature of the mechanism for the inhibition of the MSH-release. Moreover it suggests that the monoaminergic control of the MSH-cell activity is sufficient, at the hypophysial level, for the Xenopus laevis

tadpoles to be fully capable of adequate background adaptation.

It may thus be suggested that the relatively complex pattern of the innervation of the pars intermedia in adult amphibians perhaps reflects that the lobe has attained other functions besides that of colour change management. Here it should be noted that the pars intermedia in mammals (see Moriarty & Moriarty 1975) and amphibians (see Doerr-Schott 1974) participate in ACTH production. The ACTH of the rat pars intermedia is released by different stimuli (neurogenic stress) than the ACTH of the pars distalis (Moriarty & Moriarty op cit.).

General conclusions

In Xenopus laevis tadpoles the adenohypophysial rudiment gives the earliest expression of its differentiation into a secretory organ at stage 33/34. A few cells with a specific localization within the rudiment then demonstrate secretory activity. During the subsequent stages, 35/36 and 37/38, a progressively increasing amount of cells in the frontal and caudal parts of the rudiment attain the secretory state. As determined by immunohistological studies (Chapter II) the cells of the caudal part are MSH-cells. During the stages 37/38 to 39 the MSH-cells become arranged in the dorso-caudal part of the adenohypophysial rudiment, to form the pars intermedia.

According to the present study, the yolk material plays a very important role during the period of the differentiation of the secretory function of the MSH-cells. Obviously, at least two types of yolk platelets exist in the MSH-cells to meet different requirements during the initial phases of the secretory function. The most frequent type (the "unitary" type) may mainly be a source of energy, as it is disintegrated essentially without formation of any structures. About half the number of the "composite" type of yolk platelets seems to be disintegrated in a similar way. The remaining portion of the "composite" yolk

platelets, however, gives rise to several membraneous structures and is also believed to be a frequent site of de novo formation of mitochondria. The majority of the yolk platelets is disintegrated during the stages 33/34 and 35/36. The "composite" type as a rule is extinct after those stages.

Different resources for mitochondrial formation are utilized to meet the obviously acute need for mitochondrial supply during the stages 33/34 and 35/36. Thus besides the de novo formation in residual bodies of yolk platelets, mitochondria also seem to multiply by division of pre-existing mitochondria.

It may be concluded that the energy-consuming process of differentiation of the MSH-cells is passed from about stage 37/38. Thereafter only little yolk material remains, and the number of mitochondria decreases. Thereby the MSH-cells seem to pass over into a new, obviously less energy demanding state, during which cytoplasmic matter (excess or senescent) is disintegrated by lytic bodies - lysosomes and autophagic vacuoles - which are observed in increased numbers.

The synthetic activity of the MSH-cells progressively increases during all stages included in the study, in those tadpoles in which the MSH-release is uninhibited. This is manifested by increased development of the rough endoplasmic reticulum and the Golgi complexes in the MSH-cells of all tadpoles younger than about stage 40 and thereafter in those tadpoles which are kept on a black background. The reletively high rate of MSH-release in these tadpoles is shown by the MSH-containing granules characteristically occurring mainly in the surroundings of the Golgi region.

From about advanced stage 39, the pars intermedia becomes innervated by monoaminergic nerves which emerge from a heavy nerve bundle in the external zone of the infundibular floor. From the same stage the tadpoles normally become capable to adapt adequately the state of their melanophores to the background colour. The adaptation to a white background is accompanied by increased numbers of secretory

granules in the MSH-cells, also in regions distant from the Golgi region. These characters suggest that the MSH-release is inhibited, and thus indicates, as in adult amphibians, that the monoaminergic innervation is involved in the mechanism of background adaptation by means of inhibitory action on the MSH-release.

The adaptation of the tadpoles to a white background is also accompanied by a regression of the RER in the MSH-cells. This suggests that the activity of the MSH-synthesis is decreasing. As the pars intermedia of the Xenopus laevis tadpoles, with great probability, is only innervated by monoaminergic nerves, it is suggested that the activity of the MSH-synthesis is controlled by these nerves directly or via an intrinsic factor of the MSH-cells.

The cell processes of the MSH-cells, which from stage 39 frequently are observed to form dense conglomerations among the MSH-cell bodies, may promote the efficiency of the innervation, since even a few axons among them may contact processes from several cells.

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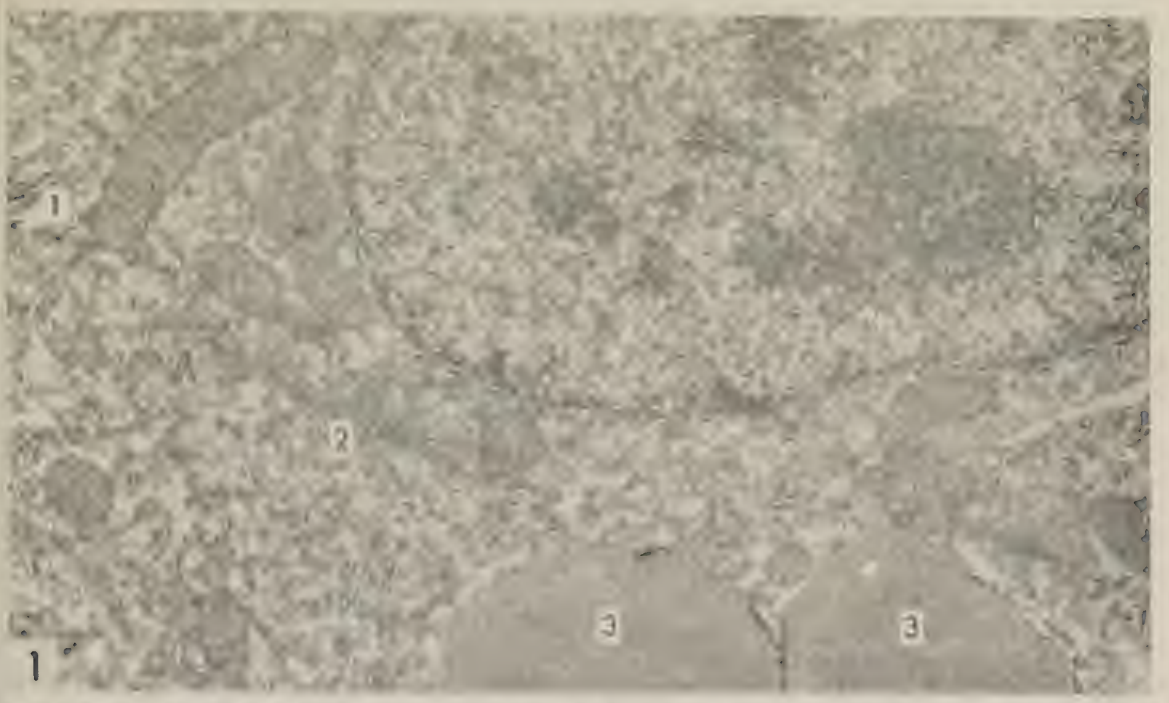
Figures III: 1 - 2.

Figure 1. Portions of adenohypophysial cells.
Stage 32. 20 400 X.

1. Rough endoplasmic reticulum
2. Golgi complex with cisternae and vesicles
3. Lipid globelet

Figure 2. Adenohypophysial secretory cells.
Stage 33/34. 20 200 X.

1. Rough endoplasmic reticulum
2. Secretory granules
3. Mitochondrion within residual body of yolk platelet
4. Residual body of yolk platelet
5. Adenohypophysis - brain interspace
6. Basal lamina of the brain
7. Axonal profile in the neuropile of the optic chiasm.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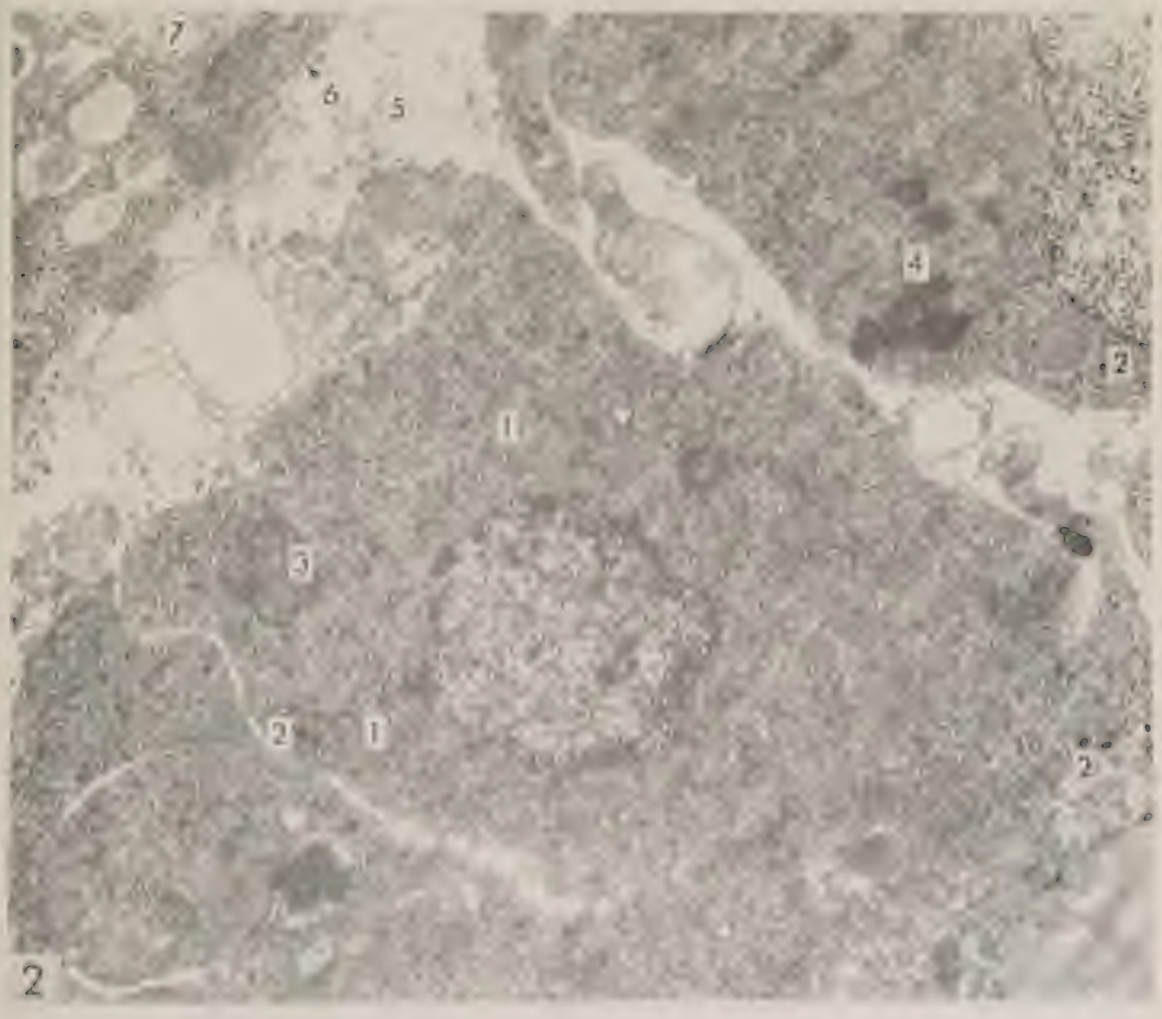
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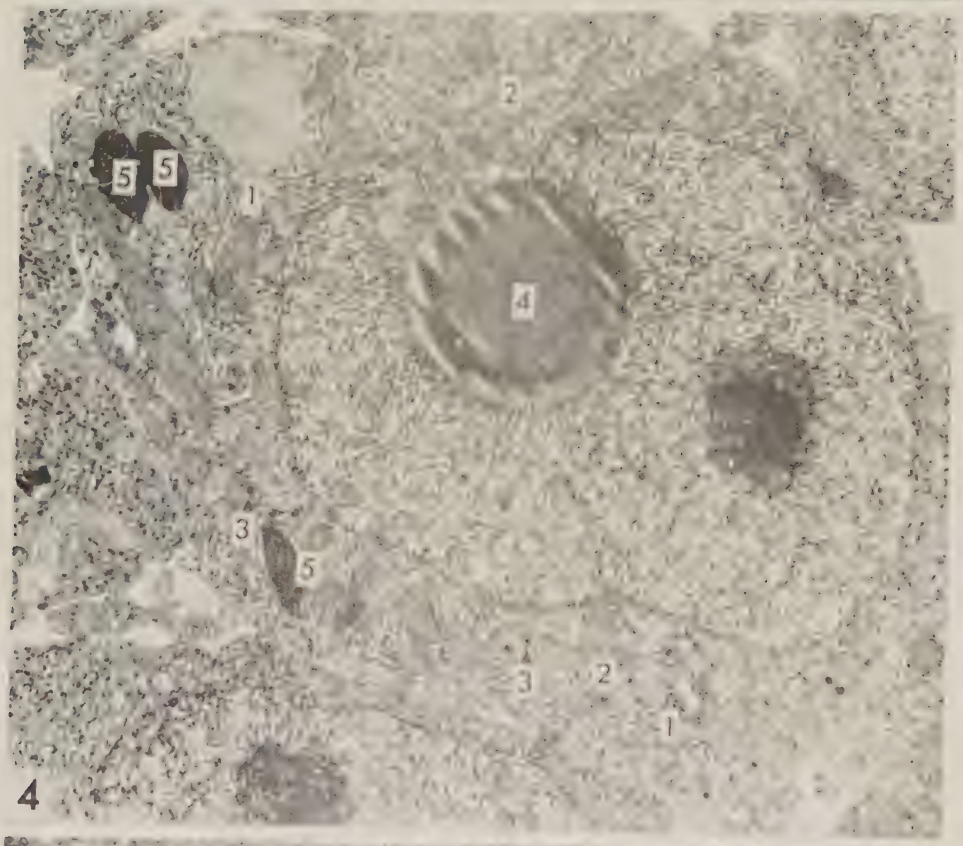

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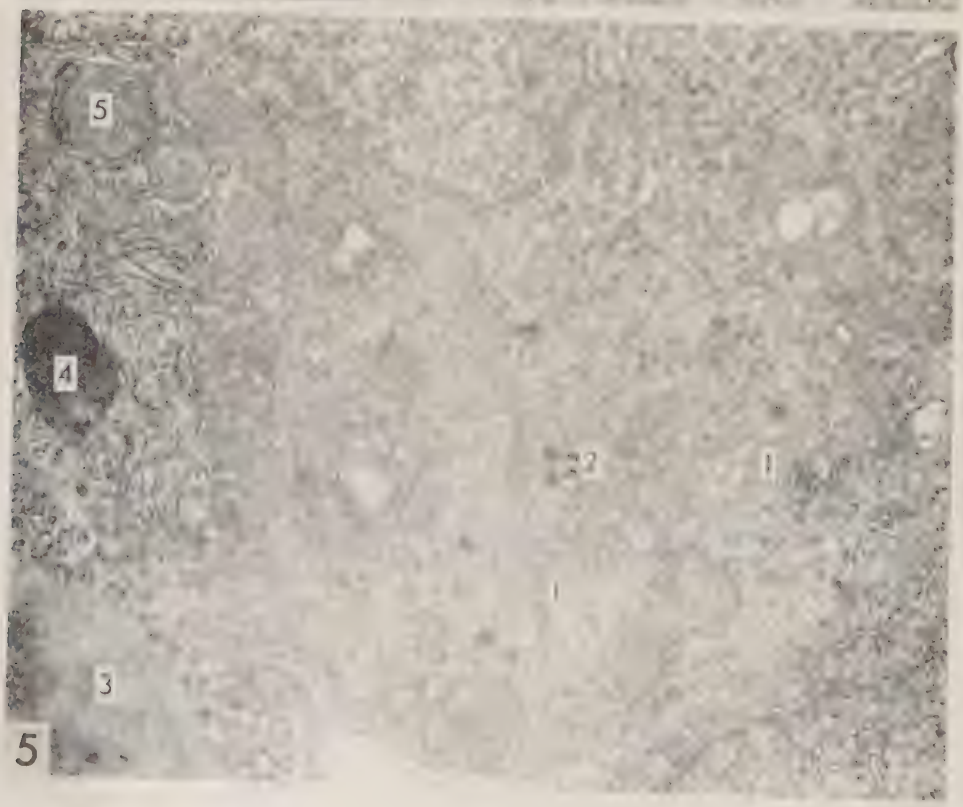

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Figures III: 3 - 5.

Figure 3. Golgi region of a secretory cell in the caudal part of the adenohypophysial rudiment. Stage 35/36. 24 600 X.

1. Rough endoplasmic reticulum
2. Golgi complex
3. Electron dense material within Golgi cisternae
4. Residual body of yolk platelet

Figure 4. MSH-cell.
Stage 37/38. 12 600 X

1. Rough endoplasmic reticulum
2. Golgi complex
3. Secretory granules
4. Lipid globelet
5. Yolk platelet

Note the high number of mitochondria.

Figure 5. Golgi region of MSH-cell.
Stage 39. Black background. 23 000 X

1. Golgi complex
2. Secretory granules
3. Lipid globelet
4. Yolk platelet
5. Formation of an autophagic vacuole - enclosure of a mitochondrion.

Figures III: 6 - 7.

Figure 6. Golgi region of a MSH-cell.

Stage 40. White background. 19 700 X

Figure 7. Golgi region of a MSH-cell.

Stage 40. Black background. 22 300 X

1. Golgi complex
2. Secretory granules
3. Cilium


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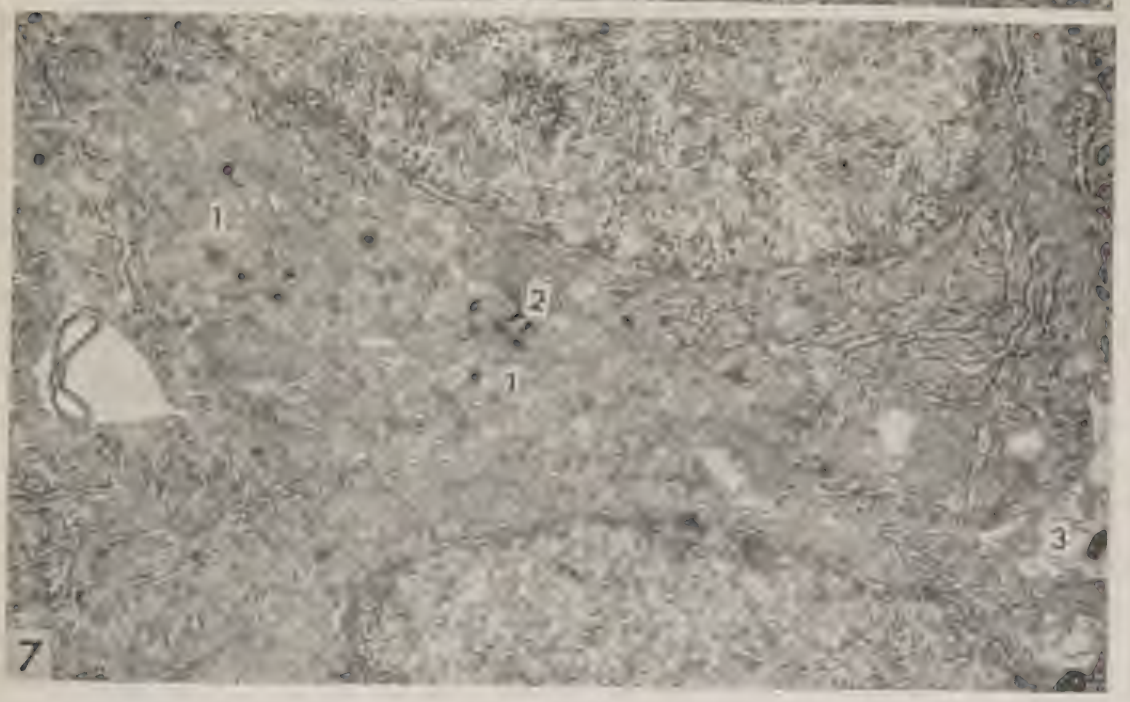
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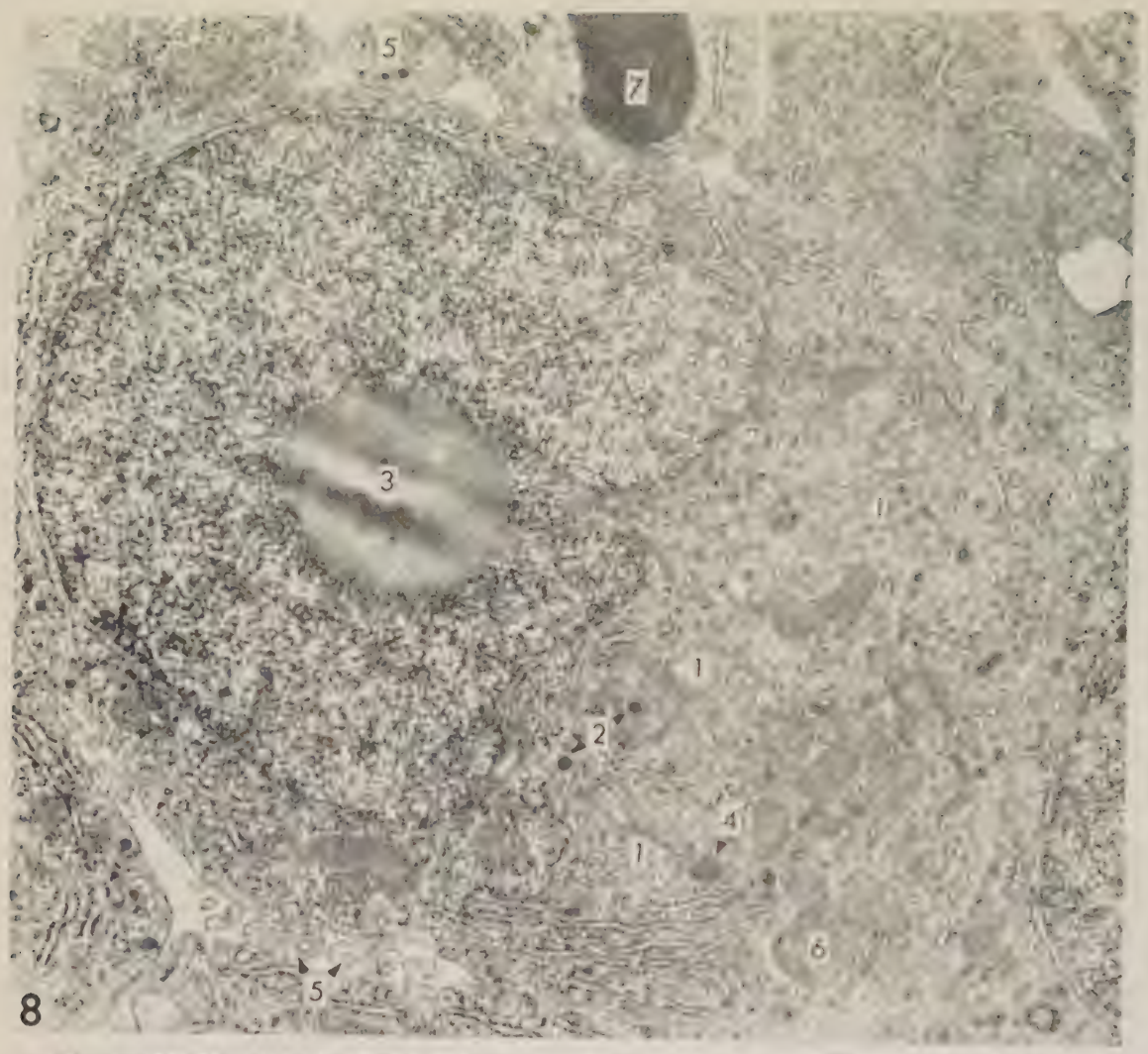
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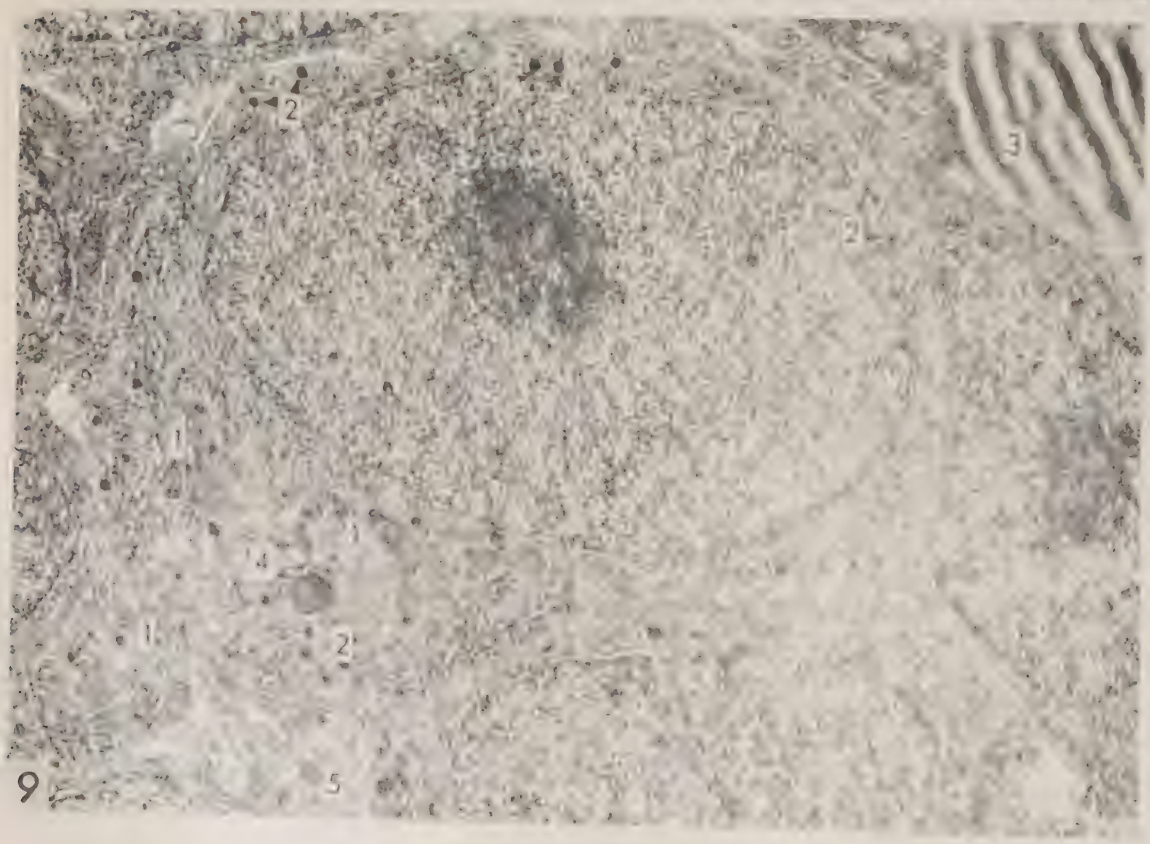
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Figures III: 8 - 9.

Figure 8. MSH-cell.

Stage 42. Black background. 19 700 X.

1. Golgi complex
2. Secretory granules
3. Lipid globelet
4. Lysosome
5. Axon
6. Formation of an autophagic vacuole
7. Yolk platelet

Figure 9. MSH-cell.

Stage 42. White background. 13 900 X

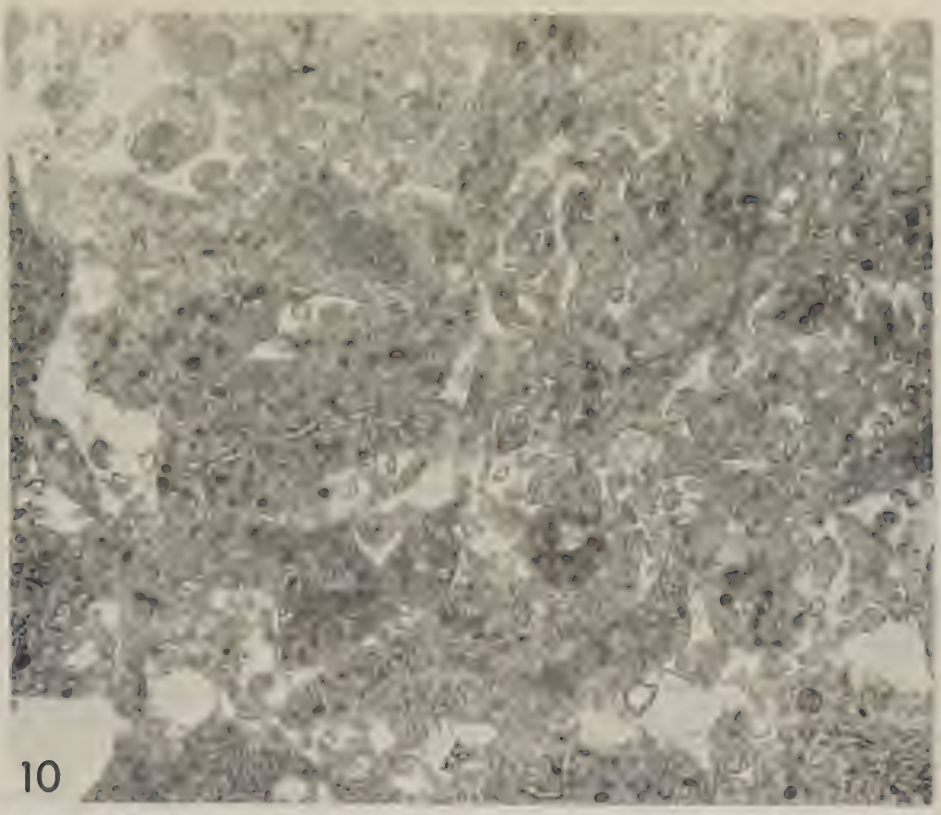
1. Golgi complex
2. Secretory granules
3. Lipid globelet
4. Lysosome
5. Axon

Figures III: 10 - 12.

Figure 10. Conglomeration of cell processes in the pars intermedia. Secretory granules and microtubules are frequent in the processes. Stage 42. White background. 25 800 X Arrow-axon.

Figure 11. Transverse section of a MSH-cell process. Stage 42. Black background. 42 200 X. Arrow-secretory granule.

Figure 12. Transverse section of a MSH-cell process containing microfilaments and microtubules. Stage 40. Black background. 46 500 X.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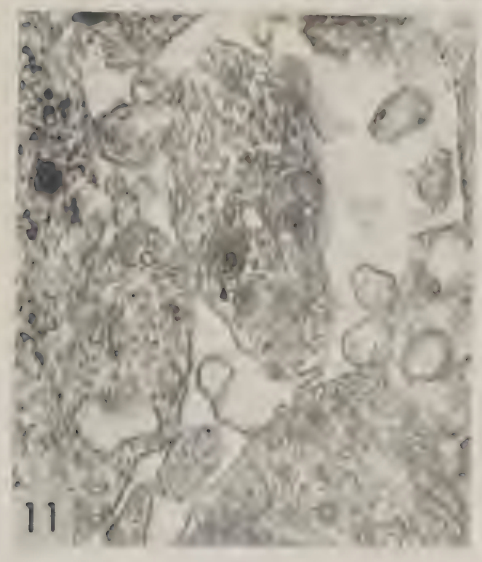
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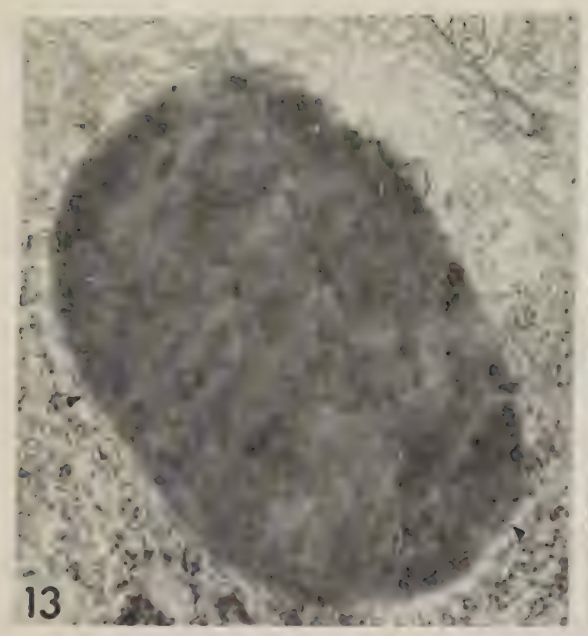
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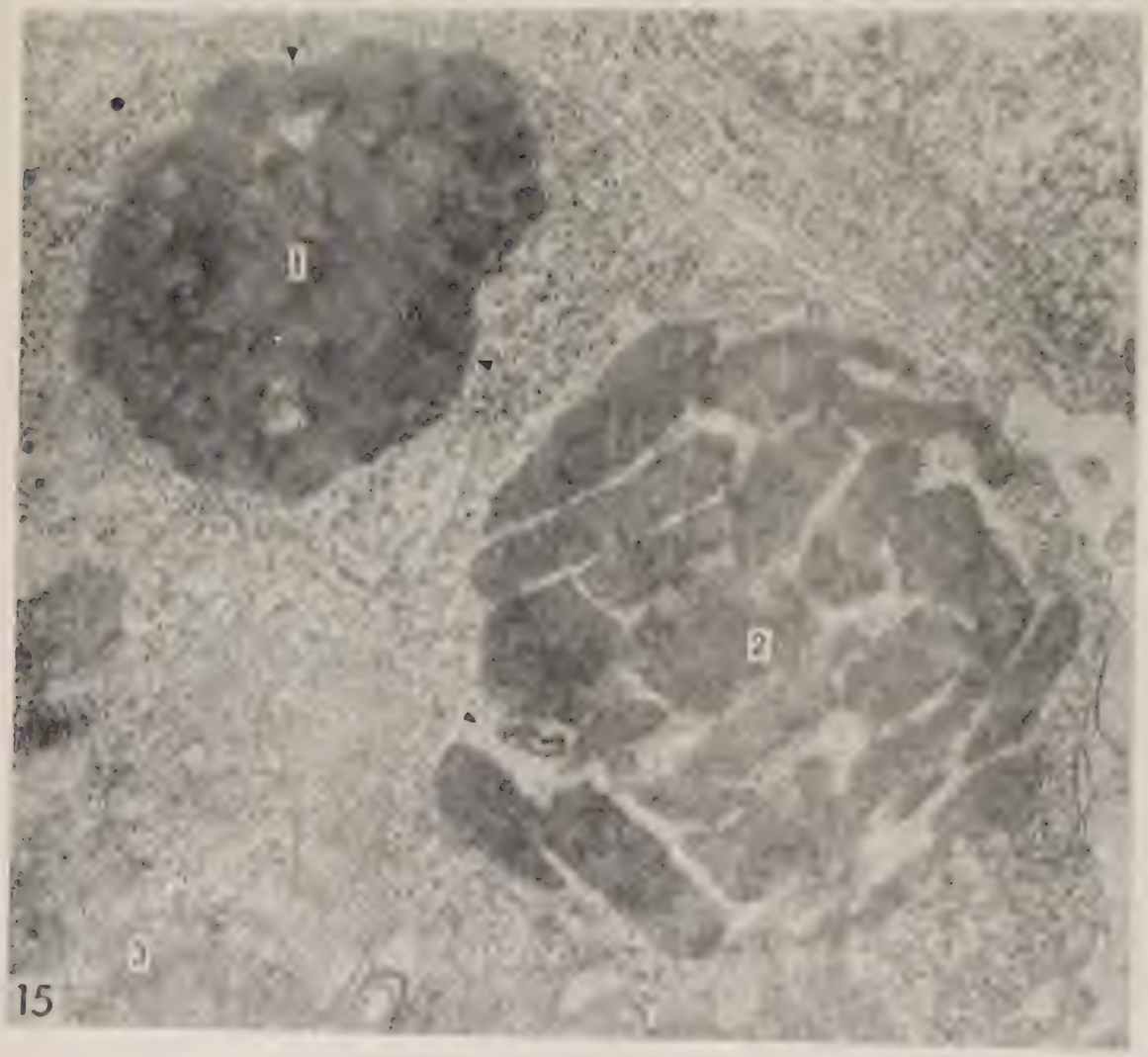
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Figures III: 13 - 15.

Figure 13. "Unitary" yolk platelet.
Stage 35/36. 43 700 X
Arrow-Limiting membrane.

Figure 14. "Unitary" yolk platelets at an advanced state of disintegration.
State 41. 38 400 X

1. Yolk platelet
2. Lysosome

Figure 15. "Composite" yolk platelets.
Stage 33/34. 43 500 X

1. Almost intact yolk platelet
2. Yolk platelet at an early state of disintegration
3. Lipid globelet

Arrow-limiting membrane of the "composite" yolk platelet.

Figures III: 16 - 17.

Figure 16. "Composite" yolk platelet which disintegrates with formation of granular material.

Stage 35/36. 68 500 X.

Arrow-limiting membrane.

Figure 17. Residual body of a yolk platelet. Patches of noncrystalline condensed matter are formed in the granular content. The limiting membrane seems intact.

Stage 35/36. 55 100 X.

Arrow: Membraneous, generally double layered, configurations in association with the dense patches.

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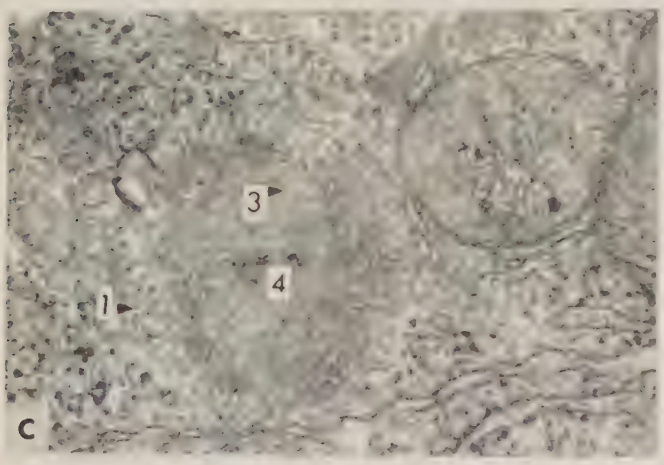
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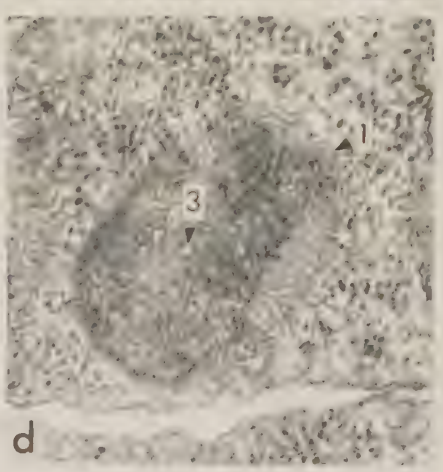
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Figures III: 18 - 19.

Figures 18 a - d. Residual bodies of yolk platelets which enclose mitochondria.

a. Stage 35/36. 51 800 X

b. Stage 35/36. 67 300 X

c. Stage 33/34. 68 500 X

d. Stage 33/34. 54 700 X

1. Limiting membrane
2. Vesicular structures in the mitochondrial matrix (see p. 11)
3. Cristae
4. Mitochondrial matrix granueles

Figure 19. Mitochondria in a state which is suggestive of division - arrow.

Stage 33/34. 34 700 X.

Figures III: 20 - 23.

Figure 20. Golgi region of MSH-cell.

Stage 42. White background. 28 400 X.

1. Golgi complex
2. Golgi smooth endoplasmic reticulum
3. "Normal" mitochondria
4. and inset. Mitochondria with amorphous matrix.

Figure 21. Golgi smooth endoplasmic reticulum with adjacent lysosome (GERL-system).

Stage 40. White background. 47 500 X.

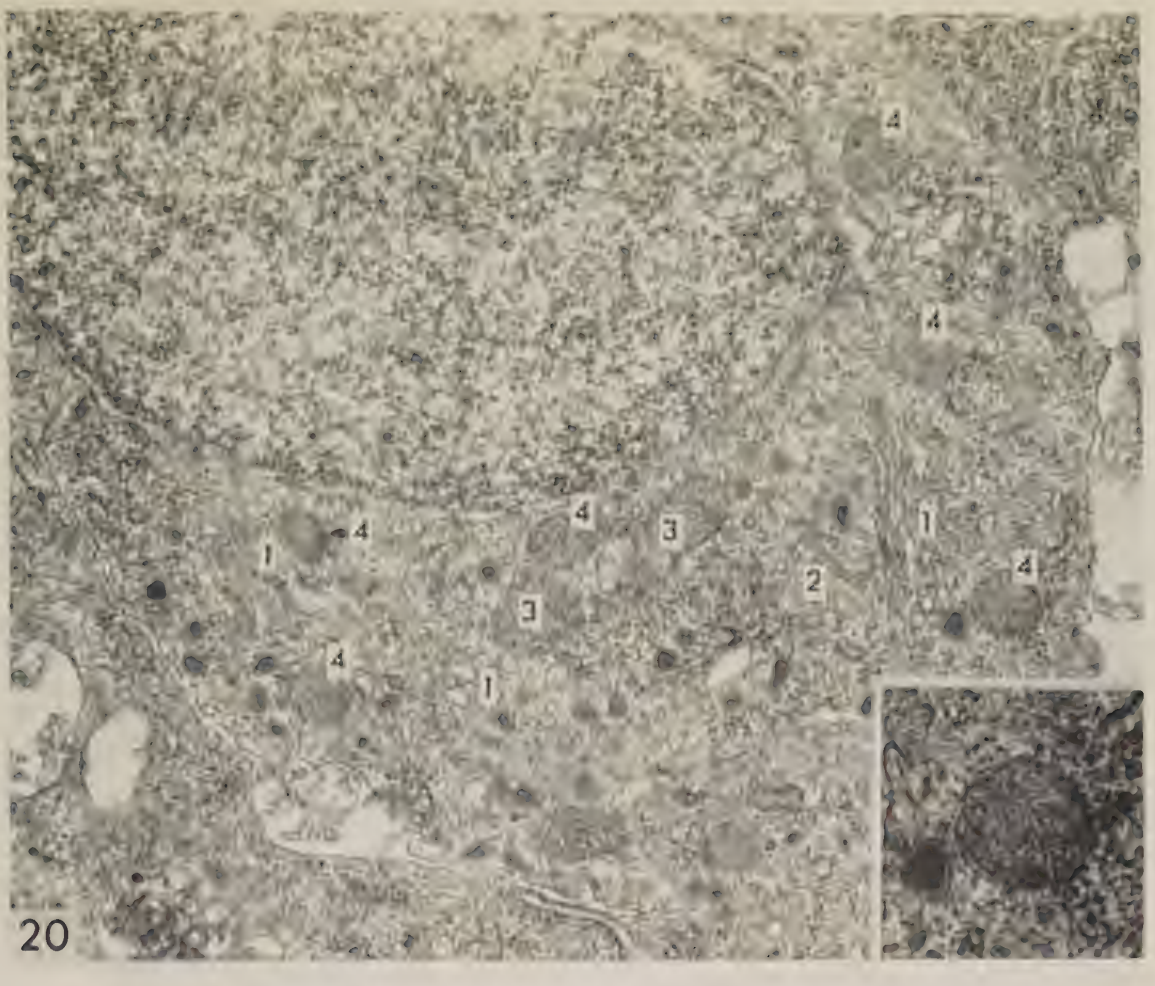
1. Golgi complex
2. Golgi endoplasmic reticulum

Figure 22. Formation of an autophagic vacuole.

Stage 42. Black background. 36 200 X.

Figure 23. Autophagic vacuole. Mitochondrion engulfed.

Stage 40. Black background. 36 500 X.



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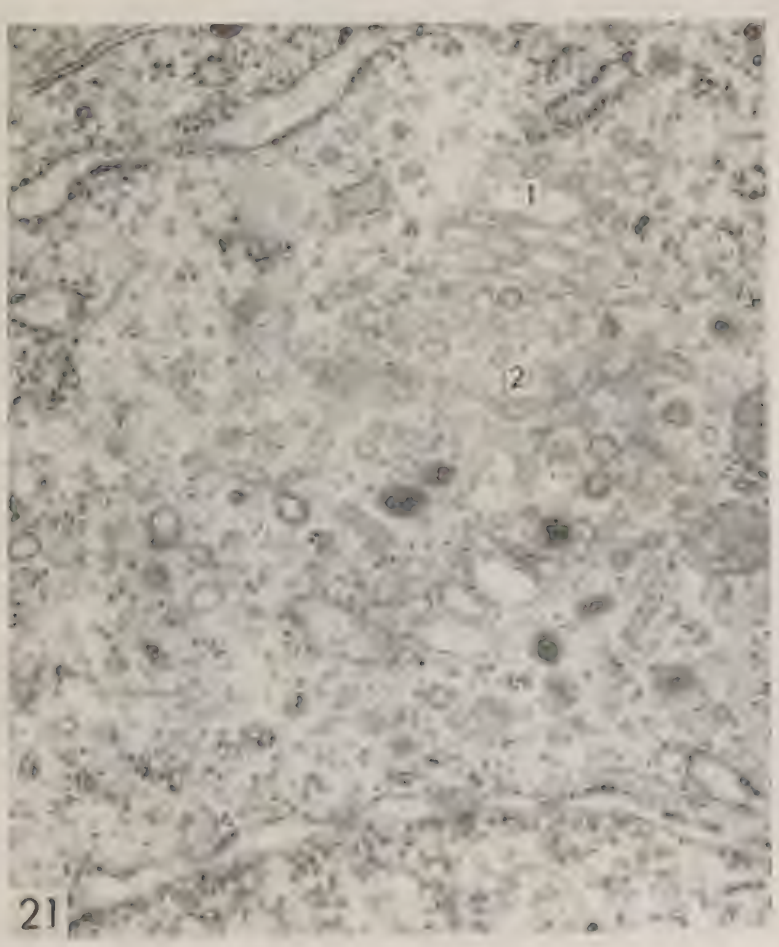

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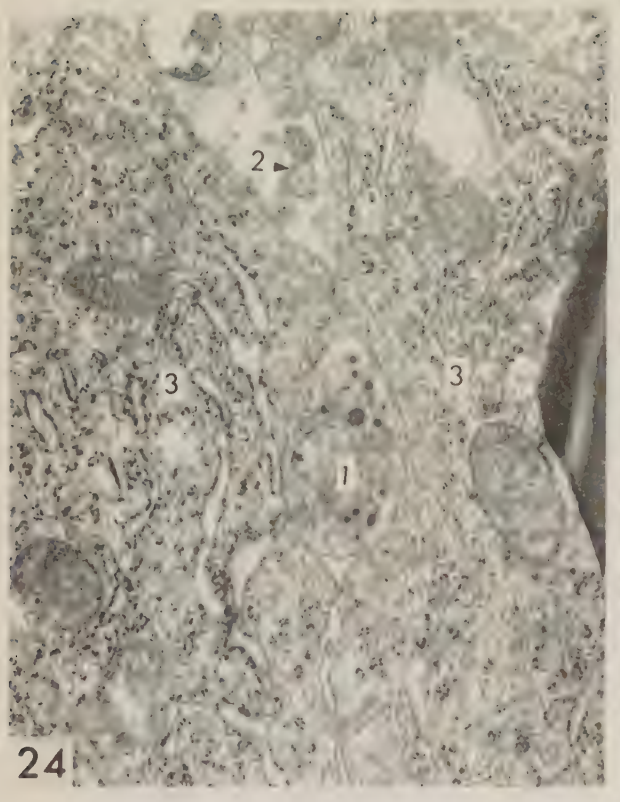

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Figures III: 24 - 26.

Figure 24. Axon in the pars intermedia.

Stage, advanced 39. Black background. 29 100 X.

1. Varicose portion of axon
2. Inter-varicose portion of axon
3. MSH-cell

Figure 25. A group of axons in the pars intermedia.

Stage 41. White background. 29 100 X.

1. Varicose portion of axon
2. Intervaricose portion of axon
3. Site of synaptic contact with MSH-cell
4. Glia cell process

Figure 26. Axons enter the adenohypophysial rudiment from the nerve bundle in the external zone of the infundibular floor.

Stage 42, Black background. 14 600 X.

1. Third ventricle
2. Nerve bundle in the external zone of the infundibular floor
3. Basal lamina of the brain
4. Cell of the adenohypophysial rudiment
5. Site of axonal penetration
6. Axons within the adenohypophysial rudiment

Figures III: 27 - 28.

Figure 27. Synaptic contact with MSH-cell process.

Stage 42. Black background. 25 700 X.

1. Axon
2. MSH-cell process

Figure 28. Axonal profiles containing large granules in the external zone of the infundibular floor.

Stage 43. White background. 25 700 X.

1. Large granule axon (peptidergic)
2. Small granule axon (monoaminergic)
3. Basal lamina of the brain
4. Fibrocyte in the adenohypophysis-brain interspace

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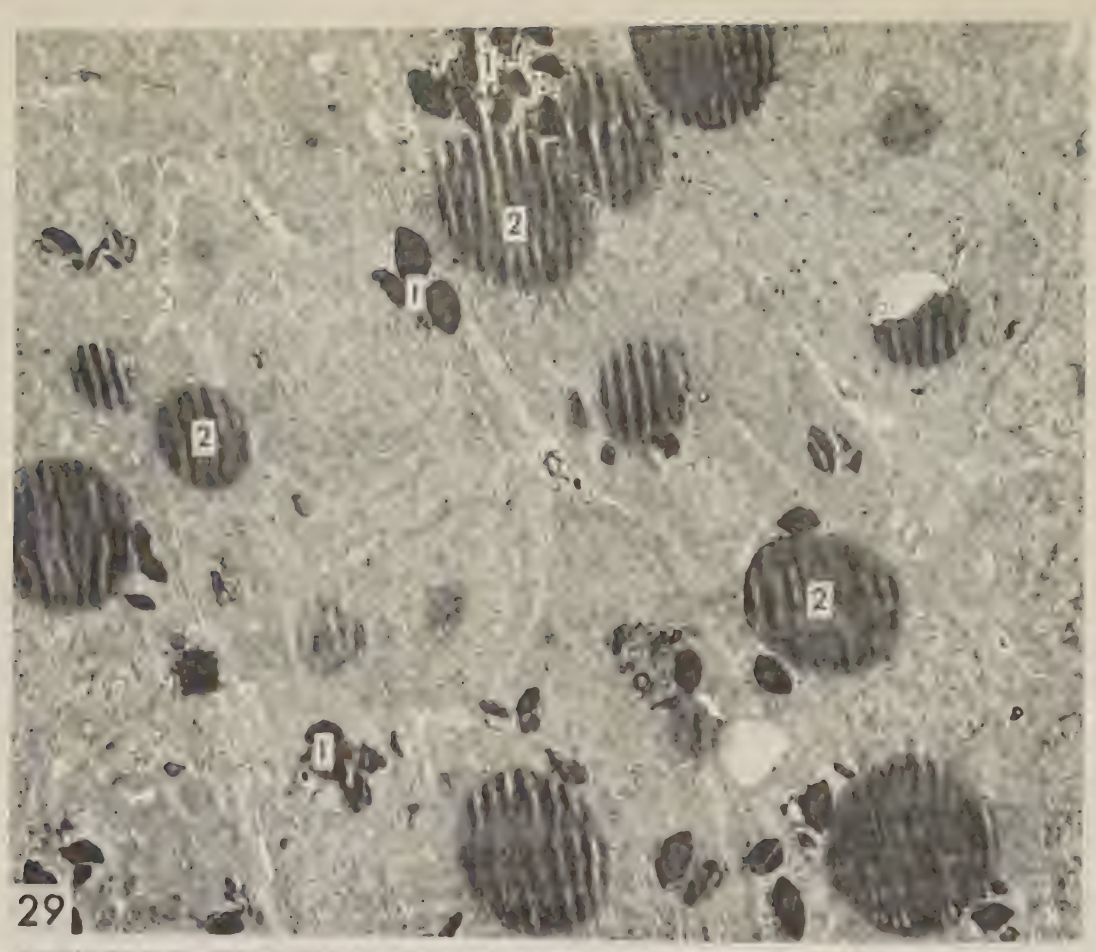

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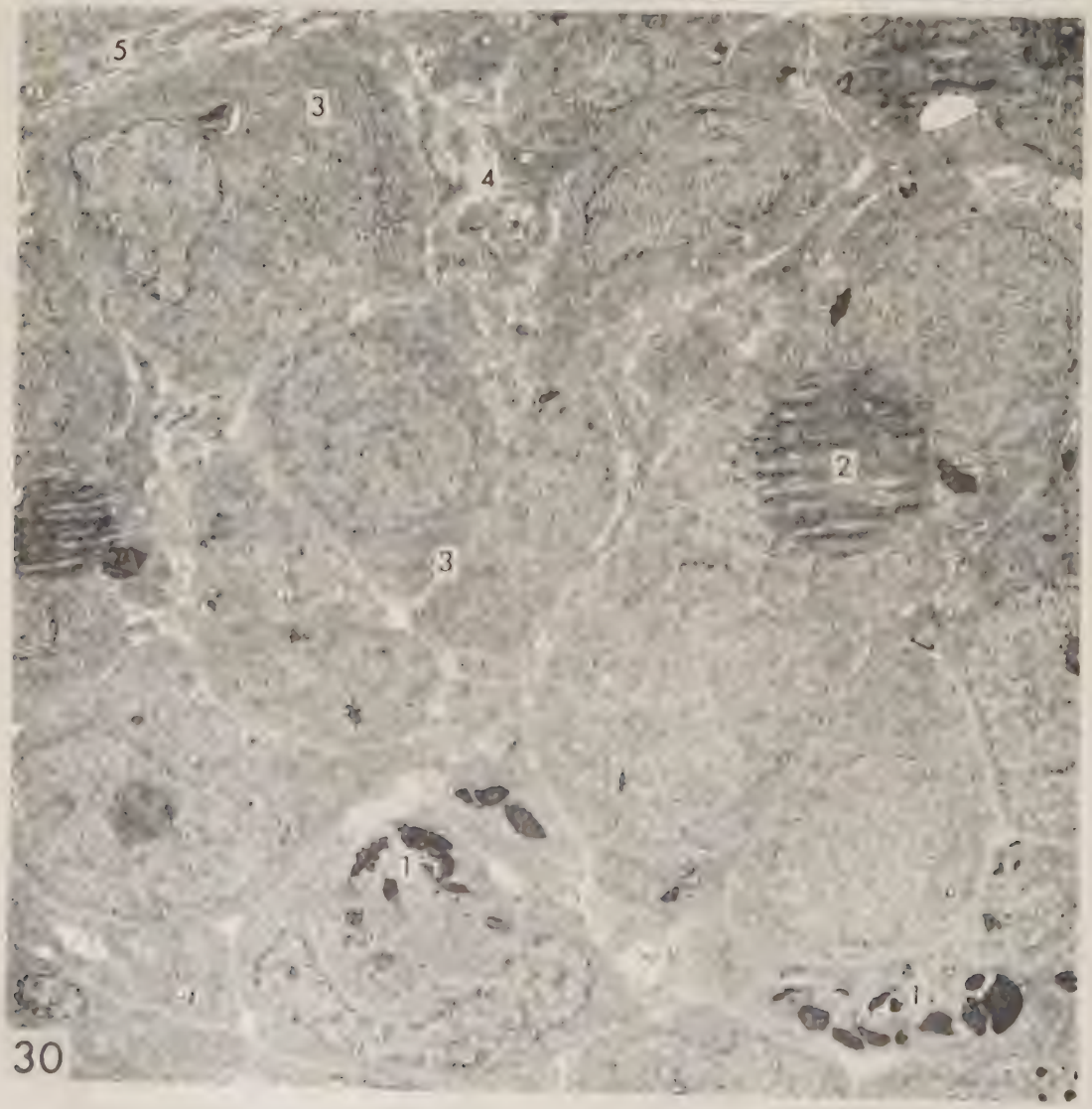

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Figures III: 29 - 30.

Figure 29. Portion of the pars distalis containing high amounts of yolk. Abnormal tadpole - cf. page 15

Stage 43. White background. 4 600 X

1. Yolk platelets
2. Lipid globelets

Figure 30. Pars intermedia of the same hypophysial rudiment as shown in fig. 29. Abnormal tadpode - cf. page 15

1. Yolk platelets
2. Lipid globelet
3. Rough endoplasmic reticulum
4. Conglomeration of MSH-cell processes
5. Border between the pars intermedia and the brain

Note the relatively low yolk content and the rich development of the RER in the pars intermedia.

Figure III: 31

Schematic summary of the yolk platelet disintegration, mitochondrial formation and degradation as suggested by the present study.

Yolk disintegration.

A. "Unitary" yolk platelets are disintegrated without formation of structures. Cf figs 13, 14

B. "Composite" yolk platelets may represent the one or the other type. The "granular" type gives rise to amorphous patches among the granular matter. Membranes are frequently observed in association with these patches. Cf figs 15, 16, 17

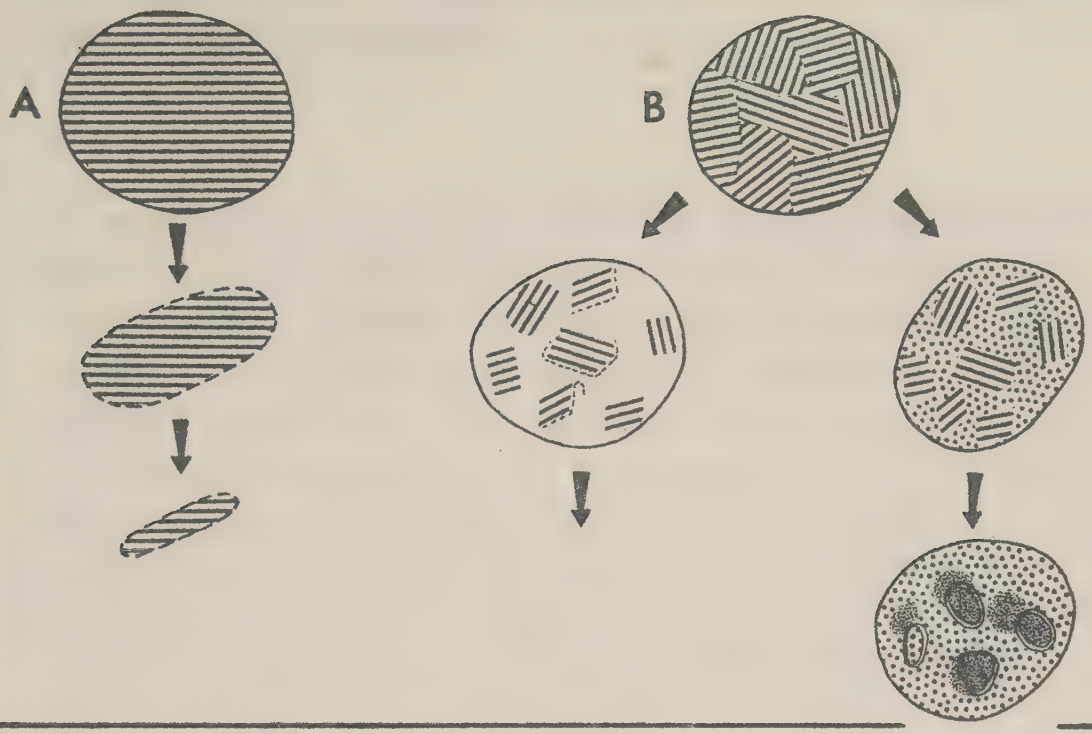
Mitochondrial formation.

Mitochondria may be formed either by division of pre-existing mitochondria or de novo from residual bodies of yolk platelets
Cf figs 18 a - d, 19

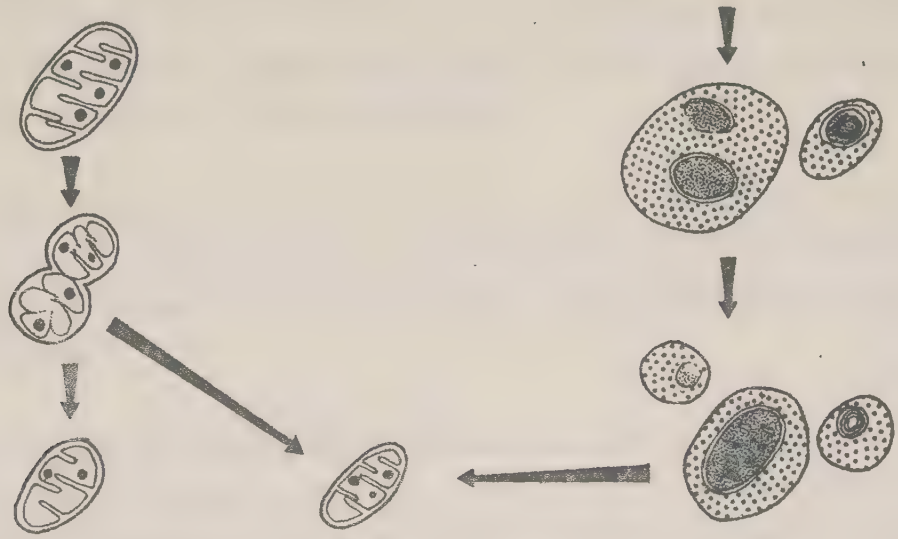
Mitochondrial degradation.

Mitochondria are observed to be engulfed by autophagic vacuoles. Cf figs 5, 23.

Yolk disintegration



Mitochondrial formation



Mitochondrial degradation

Chapter IV

THE DEVELOPMENT OF THE MELANOPHORE CONTROL IN EARLY XENOPUS LAEVIS TADPOLES

ABSTRACT

In normal Xenopus laevis tadpoles the melanin of the melanophores, as they first appear at stage 32 to 33/34, is rather concentrated. During the further development, until about stage 39, the pigment progressively disperses to maximal darkening of the skin. This "initial melanin dispersion", which occurs irrespective of the background colour, is caused by MSH-release from the adenohypophysial rudiment as revealed by it being abolished in hypophysectomized specimens. The degree of the melanin dispersion, however, is obviously limited by the ontogenetic state of the melanophores.

At advanced stage 39 to 40 the melanophore activities become background dependent, suggesting that the control mechanism of the pars intermedia has developed.

Darkness treatment and removal of the eyes gave different results as to the melanophore states after the "initial melanophore dispersion" suggesting that these conditions are not equivalent.

Transplantations of the adenohypophysial rudiment into the pronephric region were performed. These experiments indicate that the pars intermedia after early determination is capable to differentiate without contact with brain tissue.

INTRODUCTION

Since Hogben & Slome (1931) several studies have been performed on various aspects of amphibian chromatophores and colour change (cf. Parker 1948, Waring 1963, Burgers 1966, Etkin 1967, Bagnara & Hadley 1973, Terlou et al. 1974).

The eyes, the hypothalamus and the pars intermedia are indes-

pensible for the melanophore response with respect to background adaptation. Also some evidence indicating that the pineal organ may be involved in the normal colour adaptation in larval and adult Xenopus laevis is presented by Charlton (1966). (cf. Chapter V).

The preceding chapters have considered the normal development of the MSH-cells - their function and nervous control - from morphological points of view. The present chapter presents an experimental study on the development of the integratad system which controls the melanophore activity in early Xenopus laevis tadpoles.

MATERIAL & METHODS

Tadpoles of Xenopus laevis (Daudin) were obtained from adults in which spawning was induced by HCG (Gonadex, Leo, Sweden) injected into the dorsal lymph sac. The females were given about 600 I.U. and the males about 300 I.U. as a single dose (Henriques 1964).

The tadpoles were kept at room temperature. From about stage 20 they were kept in plastic dishes which were painted to provide a white or a black background. The background was illuminated by a 60 W lamp placed about 60 cm above the dishes. No food was given before stage 46.

Darkness treatment was performed in a black box with arrangements making it possible to fix the tadpoles with formalin according to Bagnara (1963), without taking them out of the container.

Extirpation and transplantation of the adenohypophysial rudiments were performed at about stage 20. The rudiments were handled with a fine glass needle. Animals in which these operations were not successful, as revealed by histological examination, served as controls.

Transplantations of the adenohypophysial rudiment was per-

formed in 89 tadpoles. Special efforts to "clean" the grafts from brain tissue were made. The grafts were implanted into the mesenchyme of the pronephros region or, as a few exceptions, into the base of the tail. The course of the M.I. variations were regularly read from about stage 33/34 to 45, when the specimens were prepared for histological examination. Fixation was performed in Bouin-Hollande Sublimée, dehydration in alcohol - xylol. Sections were cut 6 µm and stained with Azan.

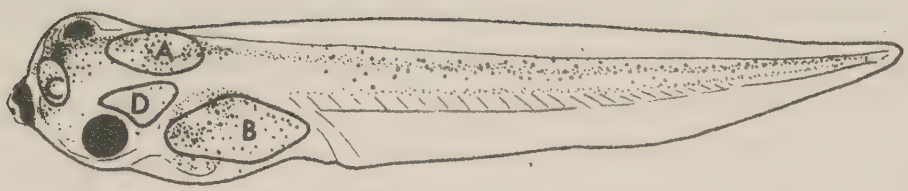
As the adenohypophysial cells at stage 45 have the same staining characteristics as have other celltypes of the grafts, the histological evaluation of the development of the hypophysial graft is very uncertain. The presence or absence of functional pars intermedia cells in the graft was therefore determined from the state of the melanophores; development of the pars intermedia function was indicated by dispersion of the melanin. Concentrated melanophore pigment indicates that pars intermedia was not developed. The tadpoles in which transplantation was performed were kept on an illuminated white background from about stage 25 to end of the experiment.

Enucleation comprised the removal of the eye rudiments on both sides at stage 29 to 31.

Animals operated at stages later than 23 were anestetized with MS 222 (Sandoz). During the operations the tadpoles were kept in position by two pins which gently pressed on their sides just behind the gill region.

The melanophore indices (M.I.) (Hogben & Slome 1931) were read in four areas of the tadpole body (Figure 1).

Figure 1. Areas in which the melanophore indices are read.



Area A - melanophores on the dorsal part of the head.

Area B - - " - the peritoneum.

Area C - - " - the frontal region, around the olfactory pit.

Area D - melanophores of the lateral head, dorsal and dorso-caudal of the eyes.

The melanophores of these areas are earliest visible at different developmental stages. In the areas A and B they appear at stage 32 to 33/34 while those of the areas C and D do not appear until stage 37/38 to 39.

RESULTS

Normal tadpoles

Constant background colour (Figure 2).

At the time for the earliest appearance of the melanophores, at stage 32 to 33/34, the melanin is rather concentrated, forming M.I. 2 to 3. During the further development the melanin undergoes a progressive dispersion until about stage 39 when the melanophores of the areas A and B show M.I. 4 to 5. Identical dispersion of the melanin occurs in the tadpoles

during the stages 33/34 to about 39, irrespective of whether they are kept on an illuminated white or black background. This background independent melanin dispersion is in the following called the initial melanin dispersion.

Stage 39 to 40 is critical as to the regulation of the melanin activity. After having passed the initial dispersion, at this stage, the melanin concentrates in those tadpoles which are kept on a white background. When continuously on a white background the concentration of the melanin is complete in all read areas about 24 hours later. In tadpoles which are kept on a black background, however, the dispersion of the melanin continues further and is complete about 24 hours later.

Changed background colour (Figures 3 a and b).

The figures 3 a and b show the effects upon the M.I. in two groups of tadpoles when the colour of the background is reversed. The animals for the experiment were all placed on a black background at stage 20 and stayed there until early stage 39. Then half the number of tadpoles were placed on a white background (fig. 3 b), while the remaining were retained on a black (fig 3 a). Irrespective of the background colour the M.I. still increased. But when the background colours again were reversed the melanin concentrated in those animals which were kept on a white background (fig. 3 a). The concentration continued as long as the animals stayed on that background. The following background reversal, to black, caused a rapid increase of the M.I.

Thus from stage 39 to 40, a change of the background colour from white to black or vice versa, is followed by distinct and adequate melanin migrations in the melanophores of normal Xenopus tadpoles (cf. Nyholm 1969 and Terlou et al. 1973).

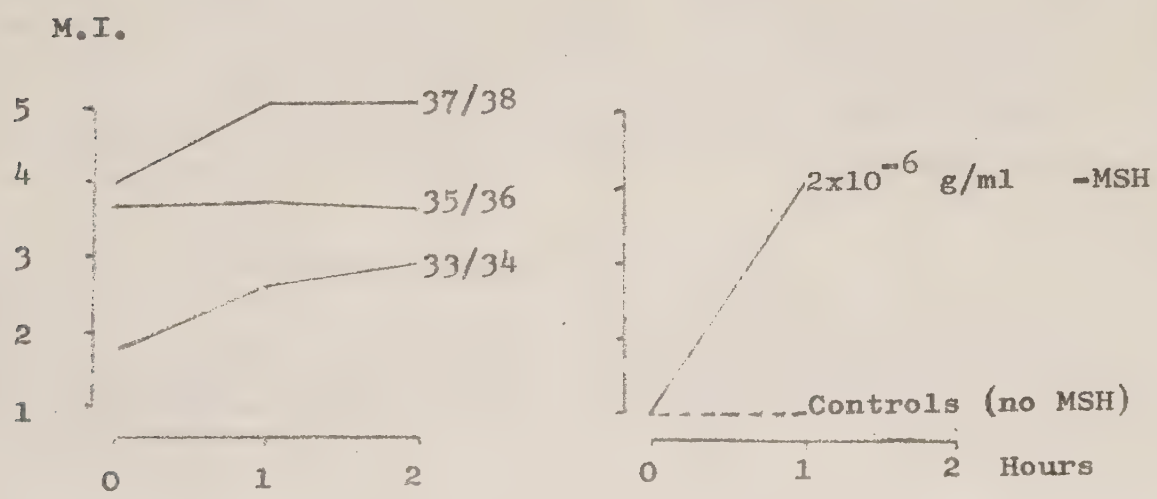
Darkness treatment (Figure 4).

The "initial melanin dispersion" can easily be recognized in tadpoles which are kept in total darkness. At stage 39 to 40 the M.I. in such animals, reaches a maximum value. The subsequent decrease continues progressively until stage 43, which is as far as studied.

Treatment with α-MSH.

Normal tadpoles at stage 33/34, 35/36 and 37/38 and tadpoles at stage 35/36 with maximally concentrated melanophore pigment due tp hypophysectomy, were exposed to 2 x 10^{-6}g α-MSH[x)] per ml in the water. The experiment was performed to test whether the M.I.:s during the "initial melanin dispersion" represent the highest attainable as directed by melanophore ontogenesis, or if they reflect increasing amounts of circulating MSH in the body fluids.

Textfigure 1 a, b. The effects upon the M.I. by treatment with α-MSH (2x10^{-6}g/ml) in the bath, in normal and hypophysectomized *Xenopus laevis* tadpoles at stage 33/34 to 37/38.



1 a. Normal tadpoles
n = 5 per stage

1 b. Hypophysectomized tadpoles, stage 35/36.
n = 3 per treatment.

x) Ciba, Bale.

It turned out that the α-MSH treatment had little effect upon the M.I. of normal tadpoles (Textfigure 1 a). In the hypophysectomized, on the other hand, the melanophore pigment dispersed by the treatment to show M.I.:s comparable with normal specimens of the same stage (Textfigure 1 b).

Operated tadpoles

Blinded tadpoles (Figure 5).

In tadpoles which are blinded by removal of the eyes, the "initial melanin dispersion" occurred as in normal tadpoles. Maximal M.I., 4 to 5, was reached at stage 39. Irrespective of the background colour the M.I. remained at this level until about stage 42, after which a minor decrease occurred on to stage 45, the last stage studied.

In conclusion, all melanin migrations in the melanophores of enucleated *Xenopus* tadpoles are background independent.

Hypophysectomized tadpoles (Figure 6).

Hypophysectomized specimens show no "initial melanin dispersion. When just visible, the melanin has the same distribution as in normal tadpoles, i.e. slightly dispersed. Very soon, however, all melanophores show totally concentrated melanin, a state which persists during the period studied.

Control animals showed melanin dispersion.

Hypohypophysectomized and control specimens were placed on a black background immediately after the operations.

Hypophysial transplantations.

The operations comprised extirpation of the adenohypophysial primordium and subsequent re-implantation of the graft, usual-

ly into the pronephros region of the same tadpole. After the operation the tadpoles were kept on a white background.

The results are summarized in table 1.

Table 1. Completeness of the extirpations and the qualities of the re-implanted grafts of the adenohypophysial primordium.

Graft development	Extirpation of the hypophysis	
	Complete[c]	Incomplete[c]
P.i.-cells functional[a]		
Brain tissue in graft	10	
No - " -	8	(46)[d]
P.i.-cells not functional[b]	7	(18)[d]
Total	25	64

p.i. - pars intermedia

a) As revealed from M.I. (= 2 - 5) after stage 39
b) - " - (= 1) - " -
c) As revealed from histological examination
d) Probable numbers, calculated from the proportions of the completely exstirpated specimens which did and did not develop functional pars intermedia cells.

In twentyfive tadpoles the exstirpations of the adenohypophysial rudiment turned out to be complete. Seven of them demonstrated pigmentory conditions identical to hypophysectomized tadpoles (cf fig. 6). This indicates that functional pars intermedia cells did not develop in the grafts of these specimens. As a rule the histological controls revealed that these grafts had developed into cysts - in two specimens, only connective tissue scars remained from the grafts.

The remaining 18, out of the 25 completely exstirpated tadpoles, demonstrated dispersed melanophore pigment (M.I. 3.5--5), which indicates that functional pars intermedia cells had developed in the grafts. Maximal M.I.:s were reached at the developmental stage 39, with the exception of two specimens which reached that state at stage 41 to 42. Thus, characteristically, the course of melanin dispersion closely resembles that of the"initial melanin dispersion"as recognized in normal tadpoles (cf. Fig. 2). No differences in this respect were observed in specimens with or without recognizable brain tissue included in the grafts.

In sixty-four of the operated tadpoles, as revealed by histological examination, some adenohypophysial tissue was retained which at stage 45 was located in the normal place - at the infundibular floor. In only a few cases did this tissue show a normal appearance - the remaining were diminished and the cellular organization often did not allow a conclusive identification of pars distalis and pars intermedia portions.

Those tadpoles in which the adenohypophysial primordium was incompletely exstirpated, all except one, showed dispersed melanophore pigment (M.I. 3-5) at the end of the experiment at stage 45. If the same proportion of the grafts did develop functional pars intermedia cells in this group as in that with completely exstirpated primordia, 46 grafts would contain functional pars intermedia cells and the remaining 18, not. This should mean that in general the tadpoles which lacked the extra pars intermedia of the graft, still could not adapt their melanophores to a white background at stage 45. An implication of this may be that the control mechanism for the MSH-release from the retained pars intermedia, at some level is disturbed by the operation.

DISCUSSION

From the earliest appearance of the melanophores in Xenopus laevis tadpoles, at stage 32 to 33/34 their pigment progressively disperses in normal specimens, but not in those which are hypophysectomized. This suggests, in agreement with Etkin (1941) and Terlou et al. (1973) that the hypophysial rudiment at these stages produces melanophore stimulating substance(s). Ultrastructural studies of the hypophysial rudiment (cf. Chapter III) also suggests that the secretory activity of the adenohypophysial rudiment starts at stage 33/34.

The "initial melanin dispersion" which takes place from stage 33/34 to about stage 39, is meantioned by Nyholm (1969, 1972) and Terlou et al. (1973)) It occurs irrespective of the colour of the background, in darkness and after removal of the light receptors - the eyes and the pineal organ (the latter cf. Chapter V). Hypophysectomy abolishes the "initial melanin dispersion", while re-implantation of the adenohypophysial rudiment restores it. Thus the "initial melanin dispersion" can be considered to reflect release of MSH (cf. Chapter II) from the adenohypophysial rudiment, which is not controlled by the environmental colour or light conditions, and is not either influenced by the eyes or the pineal organ.

The rate of the "initial melanin dispersion" seems, in normal tadpoles, to be limited by developmental conditions of the melanophores rather than by the amount of MSH in the body fluids. This is suggested by the fact that $2x10^{-5}$ g α-MSH/ml in the water did not provoke significant increase of the M.I. during the developmental stages 33/34 to 37/38. The concentration of α-MSH used, is higher than ordinarily used to study the melanophore response in isolated amphibian skin (cf. Bagnara & Hadley 1973, p. 77 and Chapter 8). The effect on the melanophores of the hypophysectomized specimens reveals that the used hormone and dose were active.

Normal Xenopus tadpoles which are kept on a white illuminated background, at advanced stage 39 to 40, abruptly begin

to exhibit an M.I. decrease, while those tadpoles which are kept on a black background continue to show increasing melanin dispersion. From this stage the normal tadpoles are also capable of adequately controlling their melanophores according to reversed white and black backgrounds. The stage for the onset of this capability coincides with that of the earliest observations of monoaminergic innervation of the pars intermedia (cf. Chapter III). As the capability of background adaptation is not developed in those tadpoles in which the adenohypophysial rudiment was transplanted, it is suggested that this innervation is functionally associated with the pars intermedia control.

The time for the attainment of the capability to adapt the melanophores according to the background colour is reached at a far less advanced developmental stage in Xenopus laevis than in other anurans studied. Etkin (1959) as related by Etkin 1967) observed that the background response in tadpoles of Rana pipiens and R. sylvestris appeared abrupt-ly, as in Xenopus but at an advanced stage. In Bufo americanus and B. terrestris (Etkin unpubl., in Etkin 1967) the tadpoles were unable to adapt their colour before the metamorphic climax.

The tadpoles which were constantly kept in total darkness, from about stage 40, demonstrated a progressively decreasing M.I. on to an intermediate state. Charlton (1966) observed the same trend of M.I. change in Xenopus laevis kept in darkness at late larval and metamorphosed stages. Franzoni et al (1972), however, reached different results in adult Triturus cristatus carnifex. In this newt the skin darkened in darkness - M.I. attaining 4 to 5. Ultrastructural examination of the pars intermedia of these specimens revealed indications of high MSH synthesis and release rates.

The blinded tadpoles of the present study showed constantly high M.I.:s after about stage 40 in three of the four read melanophore areas. The deviation of the fourth is not understood. Charlton (1966) blinded Xenopus laevis at advanced larval and at metamorphosed stages which resulted in a maxi-

mal darkening of the specimens. Imai & Takahashi (1971) likewise removed the eye balls in Xenopus laevis at advanced larval and young metamorphosed stages and in adults. All frogs became totally dark but, the older the frogs, the longer time after the operation was needed before the total darkening of the skin was obtained. This difference between young and adult specimens may explain the results obtained in blinded adult Xenopus laevis by Hogben & Slome (1931). According to those authors, the adult specimens reached an intermediate skin darkening which persists for more than one year. Imai & Takahashi (op. cit.) suggests that the skin darkening after removal of the eyes is conditioned by high release of MSH from the pars intermedia, the hypopthalmic control center of which is affected by a retrogressive degeneration of the optic pathways. The relatively long time, some weeks, between the operations and the total darkening of the skin, seems consistent with this hypothesis.

The transplantations of the adenohypophysial rudiment were performed at stage 20 to 21. At that stage the rudiment is just recognizable as a wedge like bud which adheres closely to the floor of the prosencephalon. This position of the rudiment makes it difficult to get it free from adherent brain tissue. These difficulties are reflected in the small number of specimens in which no recognizable brain tissue occurred in the graft when histologically examined at stage 45. The absence of "recognizable brain tissue" means that no nerve fibres could be observed in the graft.

The present study reveals no differences as to the differentiation of functional pars intermedia cells in the adenohypophysial grafts which do, or do not, contain recognizable brain tissue. This suggests that the MSH-cells, once determined, are differentiated independently of contact with brain tissue - possibly they are self-differentiating. This also suggests that the MSH-cells are already determined at the stage - 20 to 21 - for the transplantation. These interpretations are in accordance with those of Etkin (1958) in

Rana sylvatica.

Several other studies on anurans and urodeles have given evidence indicating that the pars intermedia has no self-differentiating capacity but, in order to become functional, requires brain tissue in the graft (i.a. Blount 1945 and Hegre 1946 in Ambystoma maculatum, Eakin 1956 and Thurmond & Eakin 1959 in Hyla regilla, Pehlemann 1965 in Pelobates fuscus and Rana esculenta; for further references see Pehlemann 1965). In general these authors consider that the infundibular floor is of special importance for the induction of the amphibian pars intermedia. This, however, seems inconsistent with observations of the present study: When , at stage 33/34, the first secrotory active cells appear in the adenohypophysial rudiment, its caudal tip has just reached the frontal border of the infundibular floor. The secretory cells, which are probably MSH-cells, however, are not first differentiated in the part of the rudiment which is adjacent to the infundibulum, but far from it - in the middle part (cf. Chapter III). Moreover, even though it is possible that some brain cells are included in the transplants of the present study and that of Etkin (1958, see Etkin 1967), it seems improbable that these cells represent the infundibular floor.

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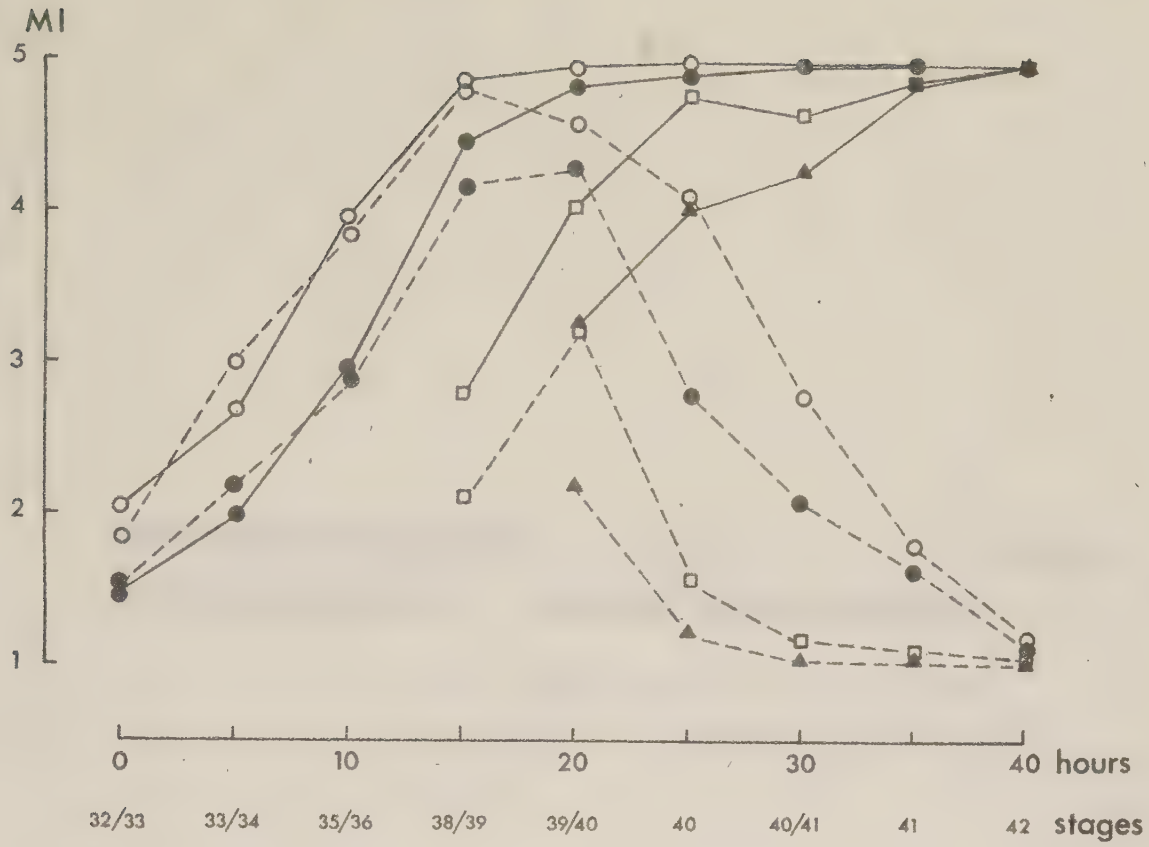


Figure IV : 2. Two groups of normal Xenopus laevis tadpoles on constant, illuminated background. From stage 32/33 a successive increase of the M.I. occurs (the period of the "initial melanin dispersion") From stage 39 to 40 the M.I. of the two groups diverge (background response).

——— = black background, ------- = white background.

● = Area A; ○ = Area B; □ = Area C; ▲ = Area D.

In each group: n = 40

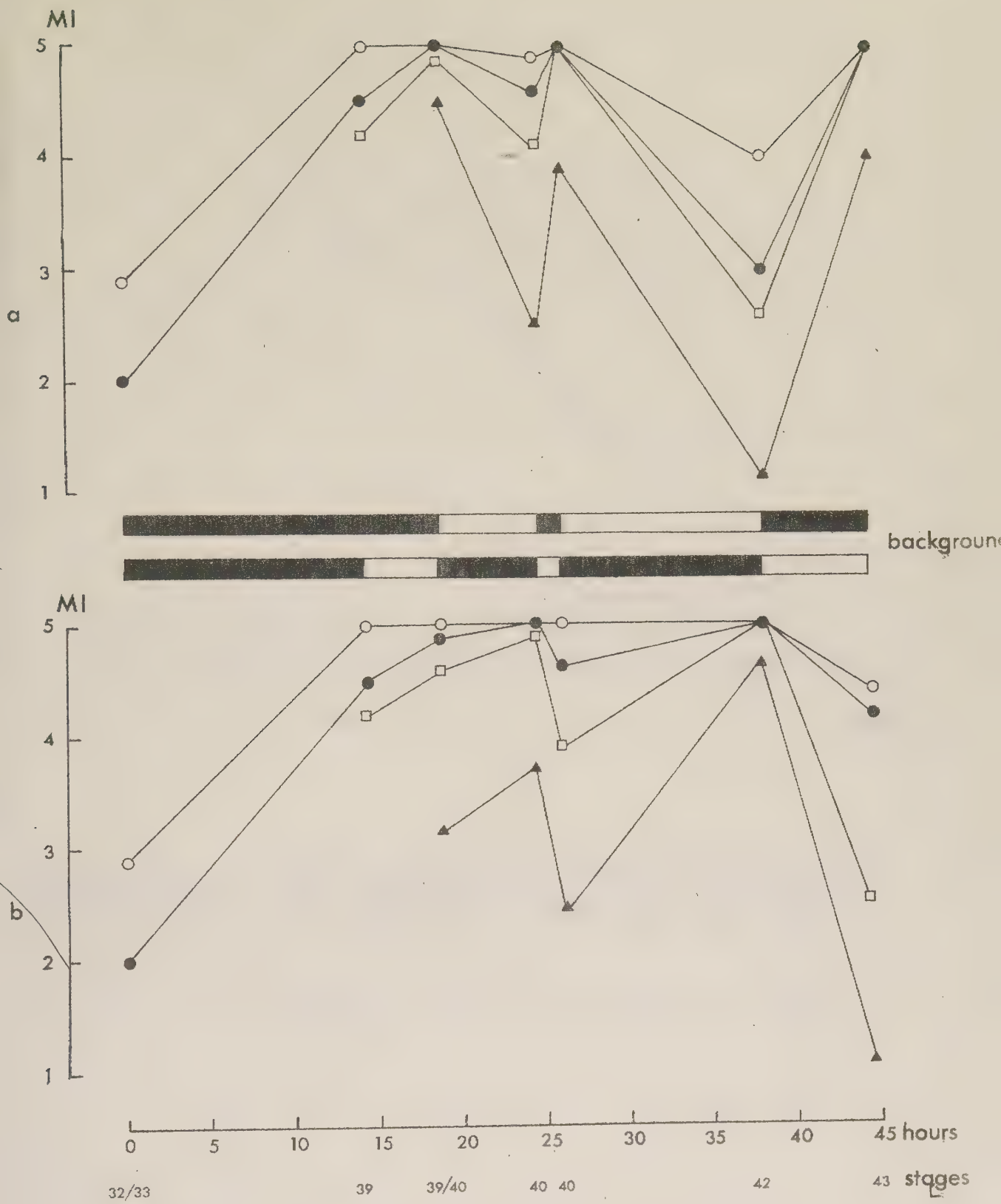


Figure IV : 3 a, b. Two groups of normal Xenopus laevis tadpoles on changed illuminated background. For legend, see p. 5

● = Area A; ○ = Area B; □ = Area C; ▲ = Area D.

In each group: n = 15.

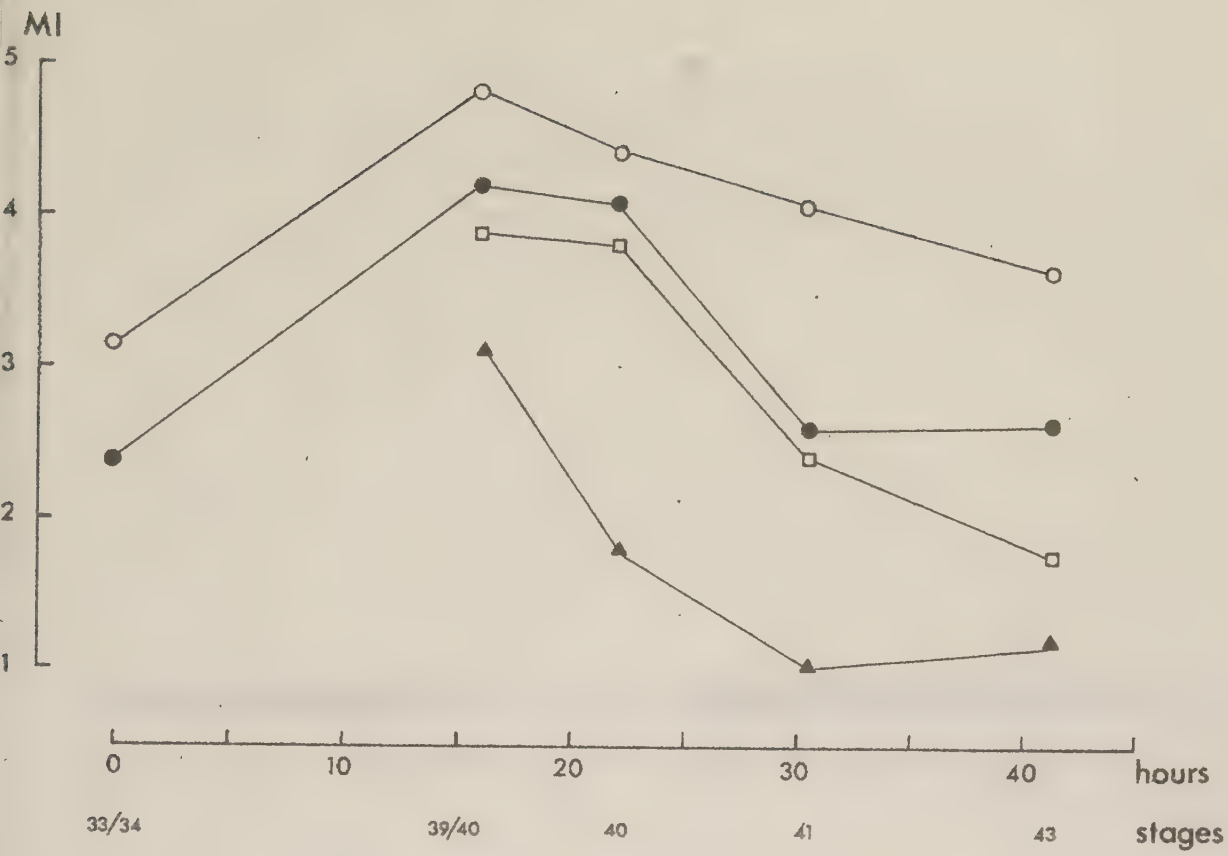


Figure IV : 4. Normal Xenopus laevis tadpoles in darkness. The "initial melanin dispersion" to stage 39, then a decreasing M.I.

● = Area A; ○ = Area B; □ = Area C; ▲ = Area D. n = 20

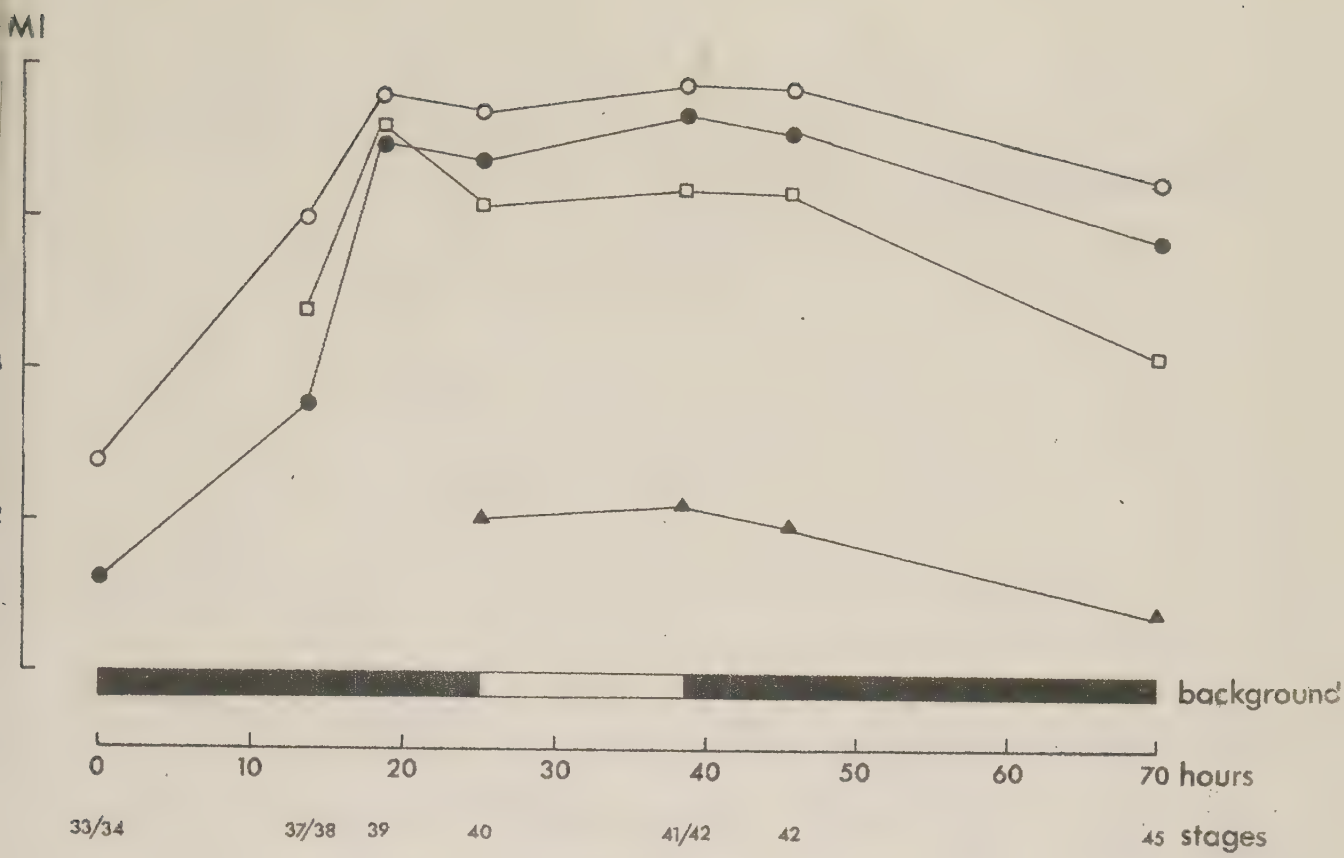


Figure IV : 5. Enucleated Xenopus laevis tadpoles on changed illuminated background. The "initial melanin dispersion" to st stage 39. Changed background colour at stage 40 and 41/42 do not cause any melanin migration.

● = Area A; ○ = Area B; □ = Area C; ▲ = Area D. n = 17

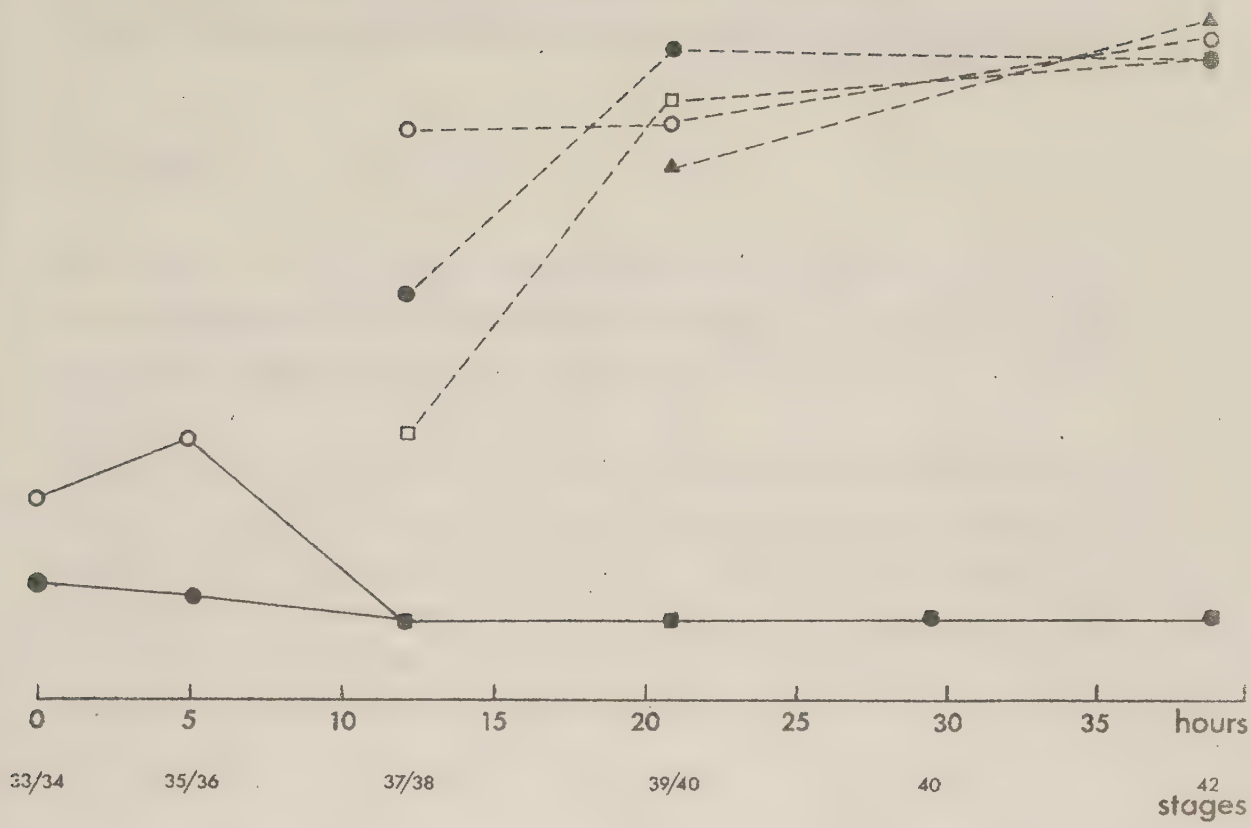


Figure IV : 6.

Hypophysectomized Xenopus laevis tadpoles (———) and controls (------) on a black background.

● = Area A; ○ = Area B; □ = Area C; ▲ = Area D.

Hypophysectomized: n = 11; controls: n = 6.

Chapter V

AN EXPERIMENTAL STUDY OF THE ROLE OF THE PINEAL ORGAN IN THE CHROMATIC RESPONSES IN EARLY XENOPUS LAEVIS TADPOLES

ABSTRACT

The role of the pineal organ for the chromatic responses - to darkness and to background colour - was investigated in early Xenopus laevis tadpoles.

Pinealectomy abolishes these responses only temporarily. They are re-established 2 to 3 days after operation, although no regeneration of pineal tissue has occurred as revealed by histological examination. This suggests that the pineal organ is not of decisive importance for the chromatic responses in amphibians.

INTRODUCTION

The current opinion of the role of the pineal organ in the control of amphibian chromatophore responses is thoroughly reviewed by Bagnara & Hadley (1973). The endocrinology of the amphibian pineal is reviewed by Bagnara & Hadley (1970).

McCord & Allen (1917) demonstrated that beef pineal extracts contained a potent concentrating agent for the melanin of the the dermal melanophores in different Rana-tadpoles. Lerner et al. (1958) identified this agent as melatonin. Much evidence suggest that also the amphibian pineal organ produces melatonin (Axelrod et al. 1965, Charlton 1966a, van de Veerdonk 1967). Baker et al. (1965) showed that melatonin also can be synthesized in other parts of the brain and in the lateral eyes of Xenopus laevis. Bagnara (see Bagnara 1965) has provided strong evidence for melatonin being responsible for the melanophore regulation in the "body blan-

ching reaction". This implies that, as a result of melatonin release in total darkness, the melanin of the dermal melanophores of amphibians strongly concentrate. From maximally dispersed melanin, the "body blanching reaction" leads to total concentration within about half an hour, in darkness. This reaction is inhibited by pineal-ectomy (Bagnara 1960, Charlton 1966b). The melatonin excerts its effect directly at the melanophore level (see Bagnara & Hadley 1970, 1973 Chapter 6).

Charlton (1966b) presents indications that the pineal organ is also involved in the background reaction of the melanophores in Xenopus laevis.

In the amphibian pineal organ, photoreceptors occur resembling those of the retina (Kelly 1962). Oshima & Gorbman (1969) suggested the the pineal organ of Rana pipiens contains initial light receptors for pars intermedia control.

In the present study the role of the pineal organ for chromatic reactions in early Xenopus laevis tadpoles, has been studied by means of pinealectomy.

MATERIAL & METHODS

Tadpoles of Xenopus laevis (Daudin) were obtained from adults in which spawning was induced by HCG (Gonadex, Leo, Sweden) injected into the dorsal lymph sac. The females were given about 600 I.U. and the males about 300 I.U. as a single dose.(Henriques 1964).

The tadpoles were kept at room temperature. For background adaptation they were kept in plastic dishes which were painted to provide a white or a black background. The background was illuminated by a 60 W lamp placed about 60 cm above the dishes. No food was given.

Pinealectomy was performed at stages 35/36, 37/38 and 42, with a fine glass needle. The skin overlying the pineal

region was removed and the pineal organ carefully lifted off. Special efforts were made to leave the brain roof as undisturbed as possible. Seventy-four totally pinealectomized tadpoles were used in the study.

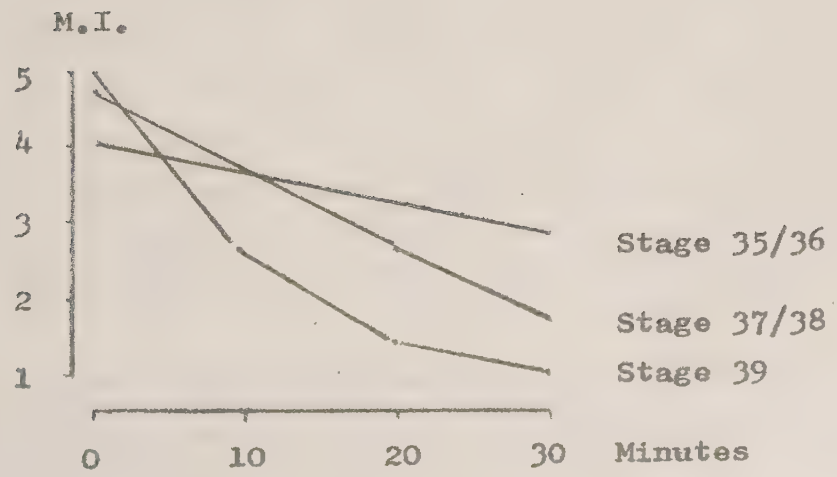
In controls the skin over the pineal region was removed and the brain roof punctured at the base of the pineal stalk.

Darkness treatment was performed in a black box.

RESULTS

The present study confirms that the "body blanching reaction" in Xenopus laevis tadpoles develops from stage 35/36 (Figure 1) (cf. Bagnara 1961). The reaction is fully pronounced at stage 39, which suggests that it is well developed when the tadpoles become capable of chromatic background adaptation (cf. Chapter IV).

Figure 1. The rates of skin lightening in darkness of intact Xenopus laevis at various developmental stages. The melanophore indices are read in Area A (cf. Chapter IV).



Pinealectomy abolishes both kinds of chromatic response and the tadpoles go dark, but only temporarily. In 2 to 3 days the "body blanching reaction" and the ability of background adaptation were re-ostablished to normal conditions in about 80 %, and partially in the remaining 20% of the tadpoles, even though histological examination reveals that no pineal tissue was regenerated.

The controls, in all respects reacted as normal tadpoles.

DISCUSSION

The current view is that the pineal organ is responsible for the "body blanching reaction" which is expressed in amphibians, in normal as well as in such devoid of their lateral eyes, when transferred into total darkness (Bagnara & Hadley (1973). The pineal organ has the qualities consistent with this view - it has its own photoreceptors (see Kelly 1962) which are activated by darkness (Dodt & Heerd 1962), and the enzymatic capacity to synthesize melatonin (Axelrod et al 1965). Melatonin is an extremely potent melanin concentrating agent (cf. Bagnara & Hadley 1973 p. 102). Moreover, Bagnara (1963) demonstrated that the cytophysiological effects of melatonin upon the dermal melanophores are similar to those which take place in the "body blanching reaction". The view that the melanin concentrating agent originates from the pineal region gaines support from observations of the melanophores on the dorsal head (Area A), which are adjacent to that region. During the onset of the "body blanching reaction" the melanin of that area concentrate more rapidly than those of other parts of the tadpole body (cf. Bagnara 1963).

The present study confirmes that the removal of the pineal organ abolishes the "body blanching reaction", but only temporarily.

Charlton (1966b) observed that the effect of pinealectomy was reduced after 2 weeks or more after operation, in advanced larval and adult Xenopus laevis. He suggests, after histological examination, that the return to normal chromatic reactions is due to regeneration of the pineal organ. The present study reveals that the normal "body blanching reaction" can be re-established as early as 2 to 3 days after operation in early Xenopus laevis tadpoles, although histological examination show that the pineal is totally removed and no signs of regeneration has occurred. This suggests that with the present method of operation, cells of the pineal "field" are retained, which have the competence to differentiate into active pineal cells. If so, they differentiate without formation of pineal structures recognizable with common histology.

An other suggestion could be that a different functional system in the neighbourhood of the pineal is critical for the chromatic responses in amphibians. This suggestion is not contradictory to available evidence.

The results by Bagnara (1960 , 1963) indicating the significance of the pineal for the "body blanching reaction" were mainly obtained in Xenopus tadpoles the pineal activity of which was abolished by heat or cold cauterization, or by removal of the dorsal prosencephalon (Bagnara 1963). Heat cauterization was also performed by Charlton (1966b) in Xenopus tadpoles. These methods seem, to the present author, to be ill-defined with regard to the sphere of action. Bagnara (cf. Bagnara & Hadley 1973) seems to be of the same opinion - as he uses the word "pinealectomy" with quotation marks. The present method of pinealectomy is to my opinion relatively specific, even though it is very probable that the operation gives rise to e.g. some traumatic effects on the adjacent region. The different times required for restoration of normal chromatic responses in Xenopus laevis tadpoles in the present study (2 to 3 days) and in that of Charlton (1966b) (2 weeks or more)

may be a matter of operational damage of the tissue adjacent to the pineal organ. Bagnara (1963) found that Xenopus tadpoles which were deprived of the whole top of the prosencephalon at an early developmental stage, subsequently were unable to blanch.

In adult Rana esculenta Bogenschütz (1967) found that epiphysectomy did not prevent the frogs from becoming pale when transferred from a black to a white background. This is in agreement with the great majority of the successfully pinealectomized specimens of the present study. After 2 to 3 days of recovery from the operation, these were able to adapt to the background in a normal way.

Oshima & Gorbman (1969) demonstrated two types of spontaneously active electrical units in the pars intermedia of adult Rana pipiens. One of these units was light-inhibitable, the other light-indifferent. The former was almost abolished in specimens which were pinealectomized. The findings by Bogenschütz (1967), however, suggest that if light-inhibitable units are significant for the chromatic responses of the frog, they do not originate from the pineal organ. Extra-pineal photo-receptor (so called "deep diencephalic photoreceptor") is evidenced by von Frisch (1911) and Scharrer (1928) in Phoxinus phoxinus. In the same species Oksche & Hartwig (1975) were able to demonstrate a circumscribed ependymal area of the third ventricle containing a photosensitive compound. Excistence of "deep" photoreceptors in the brain of amphibians are, however, not yet demonstrated.

In conclusion, the present study does not rule out that the pineal organ is involved in the chromatic responses of Xenopus laevis tadpoles. But as about 80 % of the 74 tadpoles which were pinealectomized, re-established their normal chromatic responses in a few days without pineal regeneration, this study indicates that the chromatic control is mainly extra-pineal. This suggestion seems not contradictory to former studies and is partly supported by Bogenschütz

It is however possible that pineal structures participate in chromatic responses to some degree, but are easily replaced by compensatory activity of other systems.

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